DETECTION OF ANTIBIOTIC RESISTANCE AND METALLOBETALACTAMASE AMONG PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES

Dissertation submitted in

Partial fulfillment of the Regulations required for the award of

M.D. DEGREE

In

MICROBIOLOGY– BRANCH IV

The Tamil Nadu

DR. M.G.R. MEDICAL UNIVERSITY

Chennai

APRIL 2015

DECLARATION

I, Dr. Preetthi. A.R, solemnly declare that the dissertation entitled **"DETECTION OF ANTIBIOTIC RESISTANCE AND METALLOBETALACTAMASE AMONG PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES"** was done by me at Coimbatore Medical College Hospital, during the period from August 2013 to July 2014 under the guidance and supervision of **DR.A.DHANASEKARAN, M.D, DCH,** Associate Professor, Department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr. MGR Medical University towards the partial fulfilment of the requirement for the award of M.D. Degree (Branch – IV) in Microbiology.

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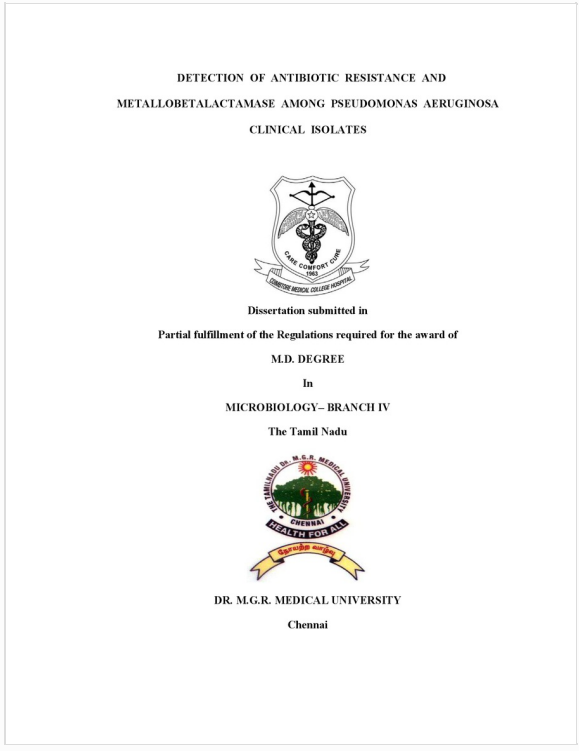

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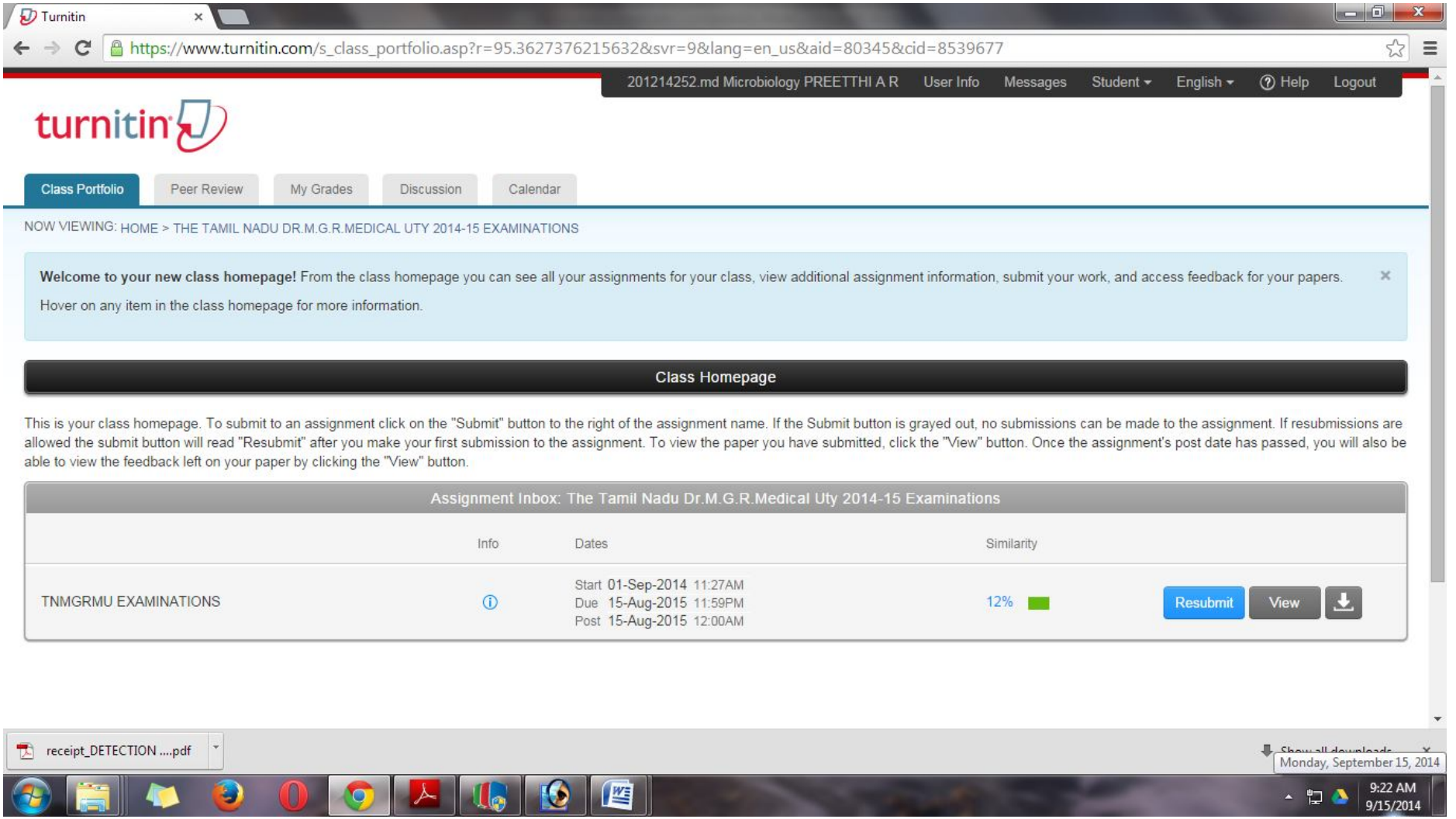
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ABSTRACT

Background And Objective: Pseudomonas aeruginosa infections are becoming an important cause of morbidity in hospitalized patients. The increasing resistance of P.aeruginosa to various antibiotics mainly carbapenems is becoming an important concern. Emergence of Metallobetalactamase producing organisms is alarming and reflects the excessive use of carbapenems . The present study was conducted at our hospital with an aim to know the prevalence of Carbapenem resistance and production of metallo-betalactamases by four different phenotypic methods.

Methodology: A total of 211 P. aeruginosa isolates from various clinical samples were tested. Antibiotic sensitivity testing was carried out by Kirby- Bauer method according to CLSI guidelines and detection of Metallobetalactamase production was carried out by Meropenem - EDTA combined disc method, Double Disc Synergy Test, Modified Hodge test and E test.

Results: Among the isolates males were more commonly affected in the above 40 years age group and the isolates were obtained from inpatients frequently. Pus swab is the predominant specimen obtained mostly from surgery ward. Cellulitis is the most observed infection. The resistance pattern to various antibiotics were- Amikacin (32.23%), Gentamycin (36.02%), Tobramycin (35.07%), Ceftazidime (71.09%),Ciprofloxacin(46.45%), Cefaperazone+sulbactum(35.07%), Cefipime(29.86%), Piperacillin+tazobactum (18.96%), Aztreonam(23.22%), Meropenem(7.58%);in urine isolates, Ofloxacin (23.8%), Norfloxacin (71.43%), Nitrofurantoin (80.95%). Among Meropenem resistant isolates, 93.75% were positive for Metallobetalactamase production detected by E test. Only 81.25% of MBL were detected by Combined Disc Test(CDT) and Double Disc Synergy Test (DDST), 75% were detected by Modified Hodge Test(MHT). E test method for MBL detection was found to have a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 100 %, 100%, 100%, 100% and 100%.

Conclusion, Significance and Impact of study: E test method is a simple and reliable method for metallobetalactamase detection which can be used routinely in any laboratory. DDST and CDT are almost equally effective and more sensitive than MHT for detection of MBL production in small laboratories which can't afford E test. Thus routine screening for MBL mediated carbapenem resistance coupled with strict infection control practices is critical to prevent the consequences of this worrying resistance mechanisms and to ensure appropriate treatment of infections caused by them in clinical settings.

Keywords- Pseudomonas aeruginosa, Metallobetalactamases, E test.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic Gram negative bacillus, motile by a single flagella and it is frequently seen in moist environment. P. aeruginosa commonly cause nosocomial infection occurring mostly in surgical wards and intensive care units .Persons with intact host defences are not at risk to get severe P. aeruginosa infection but the neutropenic patients (like patients receiving cancer chemotherapy) suffer increased risk for invasive infections. Although it can affect normal individuals, it is becoming a major threat to immunocompromised and hospitalized patients, particularly in those suffering from cancer and burns. The high mortality due to this infection is associated with bacterial resistance to antibiotics impaired host defense system.

As this bacteria is resistant to routinely used antimicrobial agents, it causes a big clinical problem and a major public health threat. The other important issue is that there are only a few antimicrobial agents including the antipseudomonal penicillins, cephalosporins, aminoglycosides, carbapenems, and fluoroquinolones

with reliable activity against it. Antimicrobial resistance in P.aeruginosa is both intrinsic[1] and acquired. **Acquired resistance**: It may be due to either chromosomal or plasmid mediated β lactamases (eg. Penicillins and cephalosporins, occasionally carbapenems and aztreonam), DNA gyrase mutation (eg. Fluoroquinolones) or decreased outer membrane permeability through porin loss (eg. Carbapenems) and aminoglycoside inactivating enzymes[2]. Because of increasing use of carbapenems in organisms producing ESBLs, now carbapenem resistant Pseudomonas aeruginosa are emerging and it leads to treatment failure even if combined drugs are being used.

There are different mechanisms for carbapenem resistance such as 1) loss of outer membrane proteins, 2) mutation in porins leading to lack of drug penetration and 3) up-regulation of efflux systems.

The important mechanism of microbial resistance is by hydrolysis of betalactam antibiotics by betalactamase enzymes. Responding to the high pressure of antibiotic use, there is continuous mutation of the genes which code for betalactamase enzymes. Thus newer betalactamases having broad spectrum of activity are being developed.

One such carbapenemase is the transferrable metallobetalactamase, a class B type of betalactamase (Ambler Classification) which recognizes bivalent metal ions mostly zinc for their catalytic activity. These metallobetalactamase has the ability to hydrolyse penicillins, cephalosporins, carbapenenems and are resistant to betalactam inhibitors and metal ion chelators like EDTA (Ethylene Diamine Tetra acetic acid), Mercapto acetic acid (MAA) and Thiol like compounds[3]. But it lacks the ability to hydrolyse aztreonam (monobactum). Metallobetalactamase producing isolates are now becoming frequently resistant to aminoglycoside and fluoroquinolones, but they are still susceptible to Polymyxin.

The genes coding for the production of Metallobetalactamases is actually a part of an integron structure[4]. These genes can either be a part of the chromosome or can be carried on tranferable plasmids. Resistance to the different groups of antimicrobial agents by P. aeruginosa isolates producing Metallobetalactamase has a potential for rapid dissemination to various types of bacteria because it is plasmid mediated.

In 1991, Pseudomonas aeruginosa producing Metallobetalactamase was being reported for the first time from Japan, and now it has spread to almost all parts of the world. Nearly 5% - 15% of the hospital acquired infections in the world are being caused by Pseudomonas aeruginosa[6]. In India Metallobetalactamase producing Pseudomonas aeruginosa are being reported from 7% - 65% in Ahmedabad[7]. Because of the emergence of antibiotic resistance in P. aeruginosa, there has been increased rate of secondary bacteremia, greater duration of hospitalization and costs and higher mortality rates.

For this scenario, widespread surveillance is required such as Implementation of simple reliable phenotypic method to detect carbapenemase and metalobetallactamase production where carbapenems and other betalactam inhibitors are used as therapeutic regimen. This will prevent their dissemination thereby initiating effective infection control measures.

As there is no single phenotypic method to detect metallobetalactamase enzymes in clinical isolates of nonfermenters even by CLSI guidelines, the most reliable phenotypic methods must be standardized. It could be possible to control the spread of resistance as well as to provide optimum

treatment to patients. Detection of gene coding for carbapenem resistance by PCR is reliable but it is of limited practical value for daily application in clinical laboratories[8]. Hence the present study was conducted with an aim to evaluate Pseudomonas aeruginosa resistance pattern to a variety of antipseudomonal antibiotics and to detect metalobetalactamase production in them using phenotypic methods such as 1) MBL E Test, 2) Combined disc test (CDT), 3) Double disc synergy test (DDST) and 4) The Modified Hodge Test. These phenotypic methods are mostly based on the ability of EDTA like metal chelators to inhibit the activity of metallobetalactamases.

AIMS AND OBJECTIVES

AIM:

To isolate Pseudomonas aeruginosa from various clinical samples and to determine its antibiotic resistance to various antipseudomonal drugs. The isolates which are resistant to meropenem are tested for various phenotypic methods for the detection of metallobetalactamase.

OBJECTIVES:

1) To isolate Pseudomonas aeruginosa from the clinical samples and to know the infections associated with it.
2) To determine their sensitivity pattern to various antipseudomonal antibiotics.
3) To correlate the meropenem resistance by disc diffusion method and its MIC by E Test method.
4) To find the prevalence of Carbapenem resistance and metallobetalactamase among the isolates.
5) To demonstrate the best and the reliable phenotypic method of metallobetalactamase production among the four tests.

REVIEW OF LITERATURE:

PSEUDOMONAS AERUGINOSA:

HISTORY:

1850: Sedillot observed blue green discharges on surgical dressings associated with infections .

1862: Luke observed rod shaped microorganism within the blue green pus.

1882: Carle Gessard first isolated the organism and designated them as Bacillus pyocyaneus.

1925: Osler discovered the organism mostly causes secondary or opportunistic infection of damaged tissues as opposed to primary infection in healthy tissues[9].

1960s: Pseudomonas aeruginosa emerged as a major human pathogen as it has the ability to cause infections in burns and in immunocompromised patients.

In Greek Pseudo means "false" and monas means "a single unit"[9], aeruginosa means verdigris that is bluish green in colour. The literal translation of blue pus is Pyocyanaea[10].

Due to the fact that Pseudomonas is a common occurrence in soil, water, plants and animals it is observed earlier in the history of microbiology. Pseudomonas is frequently present in skin and normal intestinal flora of normal man in whom it is a saprophyte. Pseudomonas is found everywhere around us: hospitals, nursing homes, medical equipments, sinks and showers in hospitals etc. It flourishes as a saprophyte in human environment in warm moist conditions such as drains, hot tubs, whirlpools, flower vases, ponds, washing machines, contact lens solutions, respirators, humidifiers, disinfectant solutions,even in distilled water[11].

MORPHOLOGY:

Pseudomonas aeruginosa is a straight or slightly curved Gram negative bacilli with a length of 1.5 to 3.0 µm and a breadth of 0.5 to 0.8 µm by . It is motile by a single flagellum inserted at the tip of the cell . Fimbriae when present are usually polar and non haemagglutinating . It is strictly aerobic. It is non acid fast, do not form spores and is non capsulated.

Many of the strains which are isolated from respiratory tract infections in patients with cystic fibrosis produce strikingly mucoid colonies.

This may be due to large amounts of alginate produced, which is an exopolysaccharide consisting of mannuronic and guluronic acids. When such strains are examined by India ink technique, the exopolysaccharide may appear as a loosely bound extracellular matrix or rarely a distinct capsule. It is to be noted that this alginate is distinct from Pseudomonas slime which is a heterogenous mixture of hexoses. It is produced on prolonged incubation in the media with low nitrogen ,high carbon content by all strains of the species. In Pseudomonas aeruginosa, filamentation cannot be induced under oxygen limitation and certain growth condition like other species of Pseudomonas[12].

CLASSIFICATION:

Classification and typing of Pseudomonas aeruginosa is being essential for epidemiological purpose because of its importance in causing hospital acquired infection. Palleroni classified Pseudomonas into five rRna homology groups which is based on ribosomal RNA – DNA homology studies. Based on this Pseudomonas aeruginosa is classified under rRNA Group 1[12].

Gilardi classified Pseudomonas into seven major groups which is based on phenotypic characteristics. Based on this Pseudomonas aeruginosa is classified under fluorescent group. It produces a water soluble pyoverdin

pigment which fluoresces white to blue green under 400nm (long wavelength) ultraviolet light and it does not accumulate poly -β- hydroxybutyrate.. Fluorescent pigment production is enhanced in media containing high phosphate concentration[13]. In earlier days, classification by serotyping, bacteriophage typing and bacteriocin (Pyocin, aeruginosin) typing were used but it is not used now due to lack of discriminatory power.

CULTURAL CHARACTERISTICS:

Pseudomonas aeruginosa, an obligate aerobe can readily grow on many types of culture media producing musty, mawkish, earthy, sweet grape like odour. (Older cultures produce corn tortilla like odour). This odour is caused by the presence of aminoacetophenone.

On nutrient agar, iridescent patches which is nothing but a moth eaten type of colonial lysis with a metallic sheen are seen with crystals beneath the patches. Though iridescence resembles lysis by bacteriophage actually there is no association with phage activity. Frequently we can observe the colony dissociation from one type of colony to another in primary diagnostic plates and in subcultures but it is not necessary that it belongs to more than one strain of species.

Blood agar plate shows the characteristic appearance that is large, mucoid, rough gray colonies with a spreading

periphery sometimes having an alligator skin appearance, and β hemolysis, oxidase production. On MacConkey Agar, Pseudomonas aeruginosa appears as pale non lactose fermenting colonies, lie flat frequently producing a gelatinous or slimy appearance, mainly in areas of heavy growth associated with pigmentation and have serrated edges. In Nutrient broth it forms surface pellicle and makes the medium turbid.

There are six different colonial types when grown on nutrient agar at 37 ° C after aerobic incubation for 24 hours.

COLONY TYPE	COLONY DESCRIPTION
TYPE 1	Large,low convex, oval with long axis in the inoculum streak line, rough in appearance, sometimes it is surrounded by a thin serrated skirt like growth. Most common and easily recognized.
TYPE 2	Small,domed,smooth in appearance,also described as coliform-like.Mostly isolated from environmental sources.
TYPE 3	Small and appear rough.
TYPE 4	Small and appear rugose.
TYPE 5	Mucoid, alginate producing,sometimes merges colonial growth. On further incubation, copious exopolysaccharide can dip on the inverted petridish lid. Mostly isolated in patients with cystic fibrosis.
TYPE 6	Dwarf type colony, sometimes appear mucoid.

Small colony variants will lack motility, require prolonged incubation time,are hyperpiliated, adhere to agar surfaces, and in liquid media show autoaggregative properties. The mucoid phenotype is also seen in occasional isolates from patients with AIDS.

There are multiple selective media containing inhibitors like acetamide, nitrofurantoin, phenenthroline, and cetrimide[14] .Selective media like cetrimide agar can be used to isolate this organism from mixed flora like in feces or other specimens. Cetrimide acts as a detergent and inhibits most of the bacteria; this medium also enhances the production of pyoverdin and pyocyanin pigment. P.aeruginosa can grow well at 37 - 42 º C; but its growth at 42 º C differentiates it from other Pseudomonas species belonging to the fluorescent group[15]. Optimum pH is 7.4 – 7.6. It can also be grown anaerobically in the presence of nitrate which acts as a terminal electron acceptor.

PRODUCTION AND IDENTIFICATION OF PIGMENTS:

PYOCYANIN AND FLUORESCEIN:

Pseudomonas aeruginosa alone produces pyocyanin, the distinctive blue pigment which is formed best in peptone media. It is water and chloroform soluble phenazine pigment which is non fluorescent. In the liquid cultures of P.aeruginosa which is grown without agitation

often we can observe a pyocyanin containing layer in the upper part of the broth at the intersurface of air / liquid. In conditions where pyocyanin presence is obscured by other pigments or it is produced in small quantity, it is observed more readily by shaking a broth culture or an agar slope culture with a few milliliters of chloroform . On standing in the chloroform pyocyanin will appear (but not fluorescein) once the phases have separated out . Pyocyanin has the capacity to emerge P.aeruginosa as the dominant bacterium in mixed infections because it can inhibit the growth of many other bacteria.

Some strains produce pyoverdin (fluorescein), a green coloured pigment, soluble in water but not in chloroform. Fluorescein is best observed in a suitable dark chamber when the cultures are illuminated with UV light. Most of the pigment enhancing media is based on King's media A and B for production of pyocyanin and fluorescein pigments respectively.

As most of the strains of P.aeruginosa has the capacity to produce both pigments, the media was developed so that one pigment production is enhanced while suppressing the other pigment production. Pyocyanin production is enhanced by Pseudomonas isolation agar in normal practice which combines the selective agent irgasan with pyocyanin enhancing properties of medium P (bacto peptone and agar, magnesium chloride,

potassium sulphate, distilled water) . Iron limitation conditions can enhance the pigment production because the pigments act as siderophores in iron uptake systems of the bacteria. Generally, non-dye containing media enhances the pigment visualisation.

PYORUBIN AND PYOMELANIN:

Pyorubin, the dark red coloured pigment and pyomelanin, a black coloured pigment are the other pigments produced by P.aeruginosa. While identifying these pigments caution should be exercised because prolonged exposure of pyocyanin to air can produce a brownish oxidation products and acidification of pyocyanin may produce a red colour. Pyorubin but not pyomelanin is produced when grown in 1% DL – glutamate. Similarly pyomelanin but not pyorubin production is enhanced in David & Mingioli's minimal salts medium which is supplemented with 1 % tyrosine.Alternatively pyomelanin production is also detected by using Furunculosis Agar. Pigment production can be enhanced by growing organism in gelatin -, potato -, or milk - containing media and by incubating them at 25 to 30 º C.

BIOCHEMICAL CHARACTERISTICS:

Pseudomonas aeruginosa is oxidase positive, catalase positive and motile. The metabolism is non fermentive and oxidative. There is no Indole and Hydrogen sulfide production. Citrate utilization test is positive. There is

no hydrolysis of urea. Methyl red reaction as well as Voges – Proskauer reactions are negative. There is oxidative utilization of glucose forming only acid. Since this weak acid produced by P.aeruginosa cannot be detected by peptone water sugars, Hugh and Leifson's medium with glucose can be used to detect the oxidative attack on sugars[16]. Xylose and fructose is also utilized oxidatively. In nitrate reduction test nitrates are reduced to nitrites and to gaseous nitrogen further. In LAO Test, L Arginine dehydrolase test is positive. L Lysine decarboxylase and L Ornithine decarboxylase test is negative. Gelatin liquefaction is produced[16] . On triple sugar iron agar, the reaction is alkaline over no change in growth.

ANTIGENIC CHARACTERISTICS:

There are 17 distinct group specific O antigens which are heat-stable and at least 2 H antigens which are heat-labile.Based on standard slide agglutination procedures, these antigens are recognised. For epidemiological typing technique only the serological characterization is primarily used. It is not used for diagnostic confirmation of species identity.

SENSITIVITY TO PHYSICAL AND CHEMICAL AGENTS:

Pseudomonas aeruginosa can be killed at 55 ° C in one hour. It can survive well in wet environments and not very resistant to drying. It exhibits a very high degree of resistance to chemical agents. It is resistant

to the following common antiseptics and disinfectants – Quarternary ammonium compounds, hexachlorophane, chloroxylenol. It can also grow in bottles of such antiseptic lotions which are kept for use in hospitals. It can pump the antiseptics actively out of the cell[17]. P.aeruginosa can also grow in cetrimide or dettol selective media. It is sensitive to the following chemicals such as acids, silver salts, beta glutaraldehyde and strong phenolic disinfectants. Silver sulphonamide compounds are used topically in burns because of its susceptibility to silver.

PATHOGENICITY AND VIRULENCE FACTORS OF PSEUDOMONAS AERUGINOSA:

Pseudomonas aeruginosa infection occurs by exposure to contaminated medical devices and solutions; introduction by penetrating wounds; ingestion of contaminated food or water; person to person transmission is assumed to occur[18]. Consuming salad vegetables which is contaminated with pseudomonas possess a very high risk for immunocompromised patients admitted in intensive care units. It produces many substances which enhance the colonization and infection of the host tissue.

Virulence Factor	Biologic Activity
Alginate	It is a capsular polysaccharide allowing the bacteria to attach to epithelial cell surfaces of lung. Can form biofilms thus protecting the bacteria from antibiotics as well as immune system.
Neuraminidase	From GM1 ganglioside receptors it removes the sialic acid residues thus facilitates the binding of pili.
Pili	Surface appendages adhere the organism to GM1 ganglioside receptors on host epithelial cell surfaces.
Lipopolysaccharide	Produces endotoxin causing sepsis syndrome.
Enterotoxin	Interrupts normal gastrointestinal activity leading to diarrhea.
Exotoxin A	Interrupts cell activity and macrophage response, causes tissue destruction,inhibits protein synthesis.
Elastase	Disrupts neutrophil activity, cleaves complement components and immunoglobulins.
Leukocidin	Inhibit lymphocyte and neutrophil function.
Phospholipase C	Destroys pulmonary surfactant, cytoplasmic membrane.Inactivates opsonins.
Pyocyanins	Disrupt respiratory ciliary activity, cause oxidative damage to tissues especially lung, suppress other bacteria.

An unusual mucoid morphotype of Pseudomonas aeruginosa is recovered from respiratory secretions of patients with cystic fibrosis. The mucoid morphotype is due to the production of alginate, a polysaccharide which surrounds the cell. Alginate is responsible for the poor prognosis and high mortality rates among cystic fibrosis patients[13].

EPIDEMIOLOGY:

Pseudomonas aeruginosa has low intrinsic virulence but approximately it accounts for 10 % of hospital acquired infections[11]. Usually in open community the carriage rate is 10-15 % but in hospital acquired the carriage rate is 30 % and the patients will start to excrete the organism in a very short period after admission. Healthy carriers will harbour the strains in the gastrointestinal tract .

TYPING SYSTEMS:

There are two different typing methods ;

1) Phenotyping methods:

The phenotyping methods are LPS serotyping and phage typing. But now, the Genotyping methods have supplanted conventional schemes which are based on phenotypic characteristics.

2) Genotyping methods .

The Genotyping methods are used for epidemiological purposes even for typing isolates from cystic fibrosis patients. But the limitation of this method is that it may not be available in all clinical diagnostic laboratories. The four different genotyping methods are :

A) RFLP:

Restriction Fragment Length Polymorphism depends upon the genetic diversity which exists upstream of the gene for exotoxin A (exo A) in P. aeruginosa. This method of typing has proved superior to all other phenotypic method for typing P. aeruginosa. Individual cystic fibrosis patients are mostly infected with same strain even when there are changes in pilin protein expression that are demonstrated by Pilin gene RFLP. Disadvantages: 1) Cumbersome nature. 2) Relatively weak discriminatory power when compared to newer methods. 3) Predominant use of two radioactive probes.

B) PFGE:

Since P. aeruginosa has substantial genetic plasticity, more than three band difference can be seen among isolates typed by Pulsed field gel Electrophoresis (PFGE) and they are considered to be of same

strain epidemiologically. This is against the Tenover's criteria which state that three or fewer band difference between two isolates should be considered from the same strain because such differences are due to only one genetic event. Advantages: 1) High discriminatory power, 2) Universal utility for typing any bacterial specimen. Disadvantages: Inability to evaluate a large number of isolates rapidly as it requires specialized equipments.

C) PCR Based Typing Methods:

They are directed against random but relatively frequent encoded sequences or at known elements within the genome. The latter method RAPD, Random Amplified Polymorphic DNA analysis used for typing P. aeruginosa has been proved quite robust. It must be consistently run to yield reproducible results on same equipment. Data from RAPD Analysis are highly consistent with the data from PFGE. To produce more discriminatory data, PCR amplification products are digested with restriction enzymes.

D) Multilocus Sequence Typing:

It has been recently only employed for typing P. aeruginosa. In this method first the special genes are amplified by PCR and then the gene products are sequenced. It is done only in very specialized centres but

it can prove relatedness among isolates by providing highly reliable data. Advantage: Highly discriminative among gene typing tool. Disadvantage: 1) Extremely time consuming 2) Very expensive[14].

BIOFILMS:

Biofilms of P. aeruginosa has the ability to cause chronic opportunistic infections mainly in the elderly and the immunocompromised patients which pose a serious problem for industrialized societies in providing medical care. The most important property of biofilms is that they show marked resistance to chemical and physical agents[19]. With traditional antibiotic therapy alone they cannot be treated effectively. Biofilms assume to have protecting function from adverse environmental factors for these bacteria. P. aeruginosa is being considered a model organism for its use in the study of antibiotic resistant bacteria as it causes nosocomial infections. It is from the treatment - resistant bacteria like P. aeruginosa, researchers try to learn more about the switch from planktonic growth to the biofilm type of growth and the molecular mechanisms involved in it. They also acquire knowledge about the quorum sensing mechanism from these bacteria. This should definitely contribute to the

development of new drugs and better management of chronically infected patients clinically.

INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA:

Pseudomonas aeruginosa infections is more prevalent among patients with burns, acute leukemia, organ transplants, cystic fibrosis, intravenous drug addiction. Urinary tract infections, lower respiratory tract infections can be severe and more life threatening in immunocompromised hosts. Infections more commonly occur at sites such as indwelling catheters, tracheostomies, the external ear, burns and weeping cutaneous wounds where there is a tendency for the moisture to accumulate. Patients with burns acquire the infection by contact spread , directly by the hands of medical staff or indirectly by contaminated apparatus. In most of the Pseudomonas aeruginosa infections symptoms and signs are related to the organs involved and are non specific. Thus the infections caused by P. aeruginosa are described below in the following order.

1) Bacteremia.

2) Bone and joint infection.

3) Central Nervous system infections.

4) Ear infections.

5) Endocarditis.

6) Eye Infections.

7) Gastrointestinal Infections.

8) Respiratory Infections.

9) Skin and soft tissue infections.

10) Urinart Tract Infections.

11) Acquired Immuno Deficiency Syndrome Related Infections [20].

Bacteremia:

P. aeruginosa is one of the cause of hospital acquired bacteremia in the intensive care units. P. aeruginosa causes 6% of all bacteremias and 75% of nosocomial bacteremias. The poor prognostic factors associated with P.aeruginosa bacteremia are granulocytopenia, septic shock, inappropriate antimicrobial therapy, and the presence of septic metastatic lesions. The mortality of P. aeruginosa bacteremia ranges from 15% - 55 % and it depends on the underlying disease, the source of infection and presence or absence of septic shock. In infants and debilitating patients, Pseudomonas aeruginosa can invade the vascular walls of the blood vessels and thus has a propensity to spread in the body.

There are two types of Pseudomonas bacteremia - Primary and Secondary.

Primary bacteremia is more common among patients with hematologic malignant neoplasms suffering from neutropenia induced by chemotherapy and in those undergoing invasive procedures. Secondary bacteremia is more commonly associated with the following infections – gastrointestinal, respiratory, indwelling intravenous catheter associated and skin and soft tissue infections.

The clinical signs and symptoms invariably similar to gram negative becteremia by other organisms. The few pathognomonic signs indicating Pseudomonas bacteremia are respiratory failure, ecthyma gangrenosum skin lesion and jaundice. These skin lesions are also associated with bacteremia - cellulitis with sharply demarcated areas which become hemorrhagic as well as necrotic, vesicles that are small and painful, maculopapular eruptions on the trunk and metastatic abscess on extremities.

Bone and Joint Infections :

P. aeruginosa cause infections of the bone and joints by either direct inoculation or by contiguous spread or by hematogenous dissemination from distant sites. The predisposing factors for these infections are diabetes mellitus, intravenous drug abuse, chronic debilitation and penetrating injury. Pseudomonas has more affinity to involve

fibrocartilginous joints of axial skeleton, cartilage, joint space, synovium and cartilaginous bone.

Sternoarticular pyoarthrosis caused by Pseudomonas occurs mainly in intravenous drug users. The erythrocyte sedimentation rate is high always and there is an accompanying leukocytosis. Synovial fluid aspiration will reveal the characteristics of the pyogenic infection. Though it is difficult to identify from gram stain of synovial fluid alone, P. aeruginosa can be easily isolated by culture.

Vertebral osteomyelitis by P. aeruginosa occurs in elderly people associated either with complicated urinary tract infections or genitourinary instrumentation or during surgery. Lumbar spine is most commonly involved. Whereas cervical spine is most commonly involved in intravenous drug users. P. aeruginosa is identified as the cause of vertebral osteomyelitis by cultural characteristics or by even histologic examination of specimens which are obtained by aspiration under fluoroscopic guidance or by needle biopsy.

During pelvic surgery or in intravenous drug users, P. aeruginosa causes infection of the pubic symphisis. There is increased erythrocyte sedimentation rate and leukocytosis is rare. Since it is difficult to distinguish osteomyelitis from osteitis pubis which is a non infectious

disease, needle aspiration or biopsy of pubic symphysis is necessary to prove infectious etiology.

P. aeruginosa being the most common cause of osteochondritis of the dorsum of the foot, after puncture wounds of foot by sharp objects like nail. The infection mostly occur in children and occasionally in adults. Because P.aeruginosa has the propensity to survive in the moist environment that occurs in the soles of running shoes which are old. Thus it proves the predilection of fibrocartilginous joints which are what exactly found in small joints of the foot. Aspiration of the infected joint may be sent for culture.

Chronic contiguous osteomyelitis arises by direct inoculation or by direct extension from the infected soft tissue overlaying the bone and least likely by hematogenous spread. This is implicated in the type of infection following a penetrating trauma, compound fracture or surgery involving the bone. These infections can also complicate peripheral vascular disease with pressure necrosis of soft tissue and skin or peripheral neuropathy or decubitus ulcers overlaying the bone. Unlike Staphylococcus aureus, Pseudomonas osteomyelitis is less destructive and more indolent.

Central Nervous System Infections:

Pseudomonas aeruginosa causes central nervous system infections (brain abscess or meningitis) either by

i) direct inoculation of the brain or sub arachnoid space secondary to surgery or penetrating head trauma,

ii) extension from infected mastoid or paranasal sinuses or from other contiguous focus.

iii) blood stream infections.

The other predisposing conditions or factors are spinal anaesthesia, previous lumbar puncture, cerebrospinal fluid leak, intraventicular shunt or reservoir and cancer of head and neck or subarachnoid space. It can cause brain abscess, meningitis when introduced by lumbar puncture. The clinical course can be from several hours to days and can be accompanied by septic shock. Occasionally Pseudomonas meningitis may develop insidiously, when it is associated especially with a contiguous site of infection or subarachnoid space cancer.

Ear infections:

Swimmer's ear or mild otitis externa is the disease of external auditory meatus caused by Pseudomonas. It is a self limiting superficial infection

which usually resolves spontaneously or responds to local treatment. It can also occur in deep sea divers (under hyperbaric conditions) which is a community acquired infection.

P. aeruginosa causes invasive (malignant) otitis externa in elderly diabetic patients and occasionally in young infants with serious underlying diseases. The pathogenesis behind this invasive necrotizing pocess is explained by the penetration of the epithelium in the floor of the lateral part of external auditory canal at the junction between cartilage and bone and invasion of underlying cartilage, soft tissue and bone. The risk factors are aural water exposure and antecedent ear irrigation.

After bypassing the tympanic membrane and middle ear, the infection enters the parotid space or retromandibuar area and then to mastoid air space and temporal bone and to the base of the skull. There i) at stylomastoid foramen, it involves the seventh cranial nerve, ii) at jugular foramen , it involves the ninth, tenth, eleventh cranial nerves, iii) at hypoglossal canal, it involves the twelfth cranial nerve. There is chance that lateral and sigmoid sinuses to be involved and further to the vascular channels at the base of the skull. The infrequent complications are meningitis and brain abscess. There is almost always a marked increase in ESR sedimentation rates.

Most often P. aeruginosa causes Chronic Suppurative Otitis Media (CSOM) and at times even in combination with other organisms.

Endocarditis:

Pseudomonas aeruginosa causes native heart valve infection in intravenous drug abusers and also causes prosthetic heart valve infections. The tricuspid valve is more frequently affected in drug addicts and then the other three valves along with mural endocardium of both atrium can also be affected. Right sided disease is subacute but the left sided disease is more acute. Diagnosis is based on positive blood cultures and appropriate clinical signs. Abnormal transesophageal or transthoracic findings are confirmatory of endocarditis.

Eye Infections:

Pseudomonas aeruginosa causes endophthalmitis and keratitis (corneal ulcer). When it is introduced by direct inoculation or hematogenous spread into the eye which is avascular having an immunologically sequestered environment, it can cause destructive, devastating and sometimes sight threatening disease. As the treatment is also complicated by the blood – eye barrier which limits the antibiotic delivery to infected intraocular structures.

Eye trauma though extremely minor can predispose to corneal infection by P. aeruginosa by causing a break in the epithelial surfaces allowing easy access of bacteria to the underlying corneal stroma .The predisposing conditions of Pseudomonas keratitis are warm, humid environments, contact lens especially soft extended wear type users, having underlying ocular conditions, patients treated with topical steroids or contaminated eye medications, coma, tracheostomy or endotracheal intubation, extensive burns, intensive care and ocular irradiation.

Pseudomonas keratitis usually starts as a small central ulcer which can spread centrifugally and involves the entire cornea part of surrounding sclera. When it penetrates more deeply into the underlying stroma involving the whole of cornea. it can cause corneal perforation within 24 - 48 hours. Pseudomonas corneal ulcer should be considered as a medical emergency because perforation into the anterior chamber will lead to a rapidly destructive intraocular infection which leads to blindness. Scrapings from the floor of the corneal ulcer are examined by Gram stain and then cultured. Antibiotic susceptibility testing is to be done for all isolates because of the increasing antimicrobial resistance in Pseudomonas corneal infections.

Pseudomonas endophthalmitis occurs as a complication of penetrating injuries, intraocular surgery, perforating corneal ulcer or bacteremia originating outside the eye. It is an acute fulminant disease which rapidly causes losss of vision. This characteristic of Pseudomonas endophthalmitis distinguishes it from other less virulent bacteria like Staphlococcus epidermidis which causes less severe form of endophthalmitis. These can involve vitreous and cause panophthalmitis. Aspiration material from anterior chamber and vitreous cavity obtained for Gram stain and culture are essential for proper management of Pseudomonas endophthalmitis .Pseudomonas keratitis, corneal ulcer infections and endophthalmitis should be approached as medical emergency because it can be fulminant and cause permanent loss of vision.

Gastrointestinal Infections:

The gastrointestinal tract is the site of primary infection and also the common port of entry for Pseudomonal blood stream infections. In young infants, it is the common cause of necrotizing enterocolitis. Typhlitis, a similar disease occurs in neutropenic cancer patients but it is polymicrobial and Pseudomonas is the most important among other bacterias. The sites involved are distal ileum,cecum,ascending colon-

proximal part. In patients with acute leukemia, necrosis,,gangrene occurs which leads to perforation and peritonotis .

Shangai fever is the historical name of syndrome which resembles the enteric fever. It presents with constipation or diarrhea, fever lasting for about 1 - 2 weeks and rash. From these cases Pseudomonas is isolated from stool yet its causative role is uncertain.

Sometimes gastrointestinal infection epidemics occur in newborns and young infants in maternity and paediatric wards due to contaminated milk feeds.

Respiratory Infections:

P.aeruginosa causes acute, bacteremic or non bacteremic pneumonia mainly in immunocompromised patients. It also causes chronic respiratory tract infection in patients with bronchiectasis and cystic fibrosis. In patients who are admitted in intensive care units, there is upper respiratory tract colonization by P. aeruginosa which is due to alteration of the buccal epithelial cells due to loss of fibronectin and loss of cells anti adhesive properties. Through aspiration of upper respiratory tract infections, patients suffering from either congestive heart failure, chronic lung disease, previous antibiotic therapy, on ventilatory or respiratory inhalation therapy are predisposed to lower respiratory tract infections. Neutropenic patients or

adults receiving mechanical ventilation are at increased risk for acquiring VAP or other pneumonias caused by P. aeruginosa[16]. Thus it is the leading cause of pneumonia in ICU patients. Even community acquired pneumonia occur in normal hosts but it is uncommonly seen.

In non bacteremic pneumonia microabscess and necrotizing alveolar septa occur but the blood vessels are not involved. But in bacteremic pneumonia mainly in neutropenic patients invasion of blood vessels occur which spread to metastatic sites of infection. In both the lung fields small, nodular, hemorrhagic lesion occur diffusely which on microscopic examination involve small blood vessels, have many bacteria, and lack leukocyte reaction. Thus they represent the pulmonary counterpart of ecthyma gangrenosum which involves the skin. The disease course is fulminant mainly in neutropenic patients and it ends fatally.

Between 3 – 18 years of age most patients with cystic fibrosis suffer from chronic lower respiratory tract infections caused by P. aeruginosa mucoid strain. In CF Patients, the infecting strain may switch from the environmental phenotype (LPS smooth, motile, nonmucoid) to CF phenotype (LPS rough, nonmoile, mucoid) during the course of infection. Leukocytosis is usually present. These infections persists for life if they are

once established and will follow a progressive waxing and waning course which are arrested by frequent exacerbations.

Skin and soft tissue Infections:

a) Ecthyma gangrenosum: This focal skin lesion may be characterized by surrounding erythema, hemorrhagic necrosis and vascular invasion by bacteria. It is sometimes associated with P. aeruginosa septicemia which is also having other skin lesions like cellulitis,deep abscess, subcutaneous nodules, bullae, vesicular or pustular skin lesions. In neutropenic patients Pseudomonas sepsis is complicated by large destructive lesions of the skin or mucous membrane. These lesions can produce gangrene of face, oropharynx, extremities or perenium.

b) Pyoderma: Primary Pseudomonas pyoderma resembles ecthyma lesions of Pseudomonas bacteremia. They can be focal or diffuse. Predisposing factors arise due to breakdown of skin secondary to trauma, severe burns, swimmer's ear, diaper area of infants, soldier's foot in tropics and neutropenia occurring secondary to cancer chemotherapy.

c) Wound infections: Wound infections don't have a characteristic appearance to distinguish it from other bacteria. But it produces a characteristic fruity odour and blue green exudate by pyocyanin pigment production which are seen more commonly on wound bandages than within

the wound itself. Any non operative or post operative wound infection can be caused by P. aeruginosa and it should be seriously considered if it is contaminated with soil, water, plant material. Occasionally nosocomial outbreak of Pseudomonas infection can occur.

d) Dermatitis: P. aeruginosa causes self limited, pruritic, diffuse, maculopaular or vesiculopustular rash associated with exposure to contaminated hot tub, spa, whirlpool. These often occur as a common source outbreak. Rashes are more pronounced in bath suit covered areas but can also be diffuse and rarely systemic symptoms occur. Usually these rashes require no specific therapy except in immunocompromised patients like patient with AIDS or neutropenia. There is extensive skin involvement and it requires systemic antimicrobial therapy.

It also causes irritating folliculitis and a necrotizing skin rash which is referred as jaccuzi or hot tub syndrome that develops in people who uses these recreation facilities.

e) Burn infection: P. aeruginosa is the most common cause of burn wound infection having a high mortality rate. Yhey occur 1 – 2 weeks after severe thermal injury. During the lag period the normal flora of the patients skin is replaced by the hospital flora of Pseudomonas mainly under the pressure of antibiotics. Sepsis will result from burn eschar colonization, invasion of

subeschar space and invasion of the bloodstream. Systemic symptoms may occur and ecthyma gangrenosum lesions appear at distant site from burn wound infection. Patient may get pneumonia if any inhalational injury has occurred.

Clinical features of burn wound infections are multifocal discoloration of eschar, granulation tissue degeneration, hemorrhagic necrosis and edema of tissue adjacent to burn site and brown to black neoeschar formation. For successful treatment early recognition of wound sepsis by daily surveillance of wound site focusing on inflammatory signs and systemic symptoms monitoring is essential. Biopsy including quantitative culture is done in suspicious skin sites. The various diagnostic features are presence of $> 10^5$ organisms per gram of tissue, masses of organisms in subeschar space, bacteria in unburned tissue, perivascular inflammation and hemorrhage at the burn wound margin.

It can also cause infection of the nail beds in people with artificial nails[21].

Urinary Tract Infections:

Urinary tract infections by Pseudomonas are hospital acquired and some are iatrogenic. The predisposing factors of UTI are catheterization, by introducing instruments, by irrigating solutions, surgery including renal

transplantation, persistent sites of infection (like chronic prostatitis or stones), obstruction or previous antibiotic therapy. Chronic Pseudomonas urinary tract infections and recurrences are commonly seen and they are complicated by multidrug resistant Pseudomonas

Pseudomonas aeruginosa in patients with AIDS:

P. aeruginosa infection are noted in people with advanced stage of Acquired Immunodeficiency Syndrome. The immunological factors like loss of mucosal integrity, defects in humoral and cellular immunity, leukocyte abnormalities render the HIV infected patients more vulnerable to life threatening P. aeruginosa infection. Mostly they occur in patients with low CD_4 count and with history of opportunistic infections. These infections occur either in presence or absence of risk factors for development of Pseudomonas infections like previous antibiotic therapy, hospitalization, indwelling vascular catheter and neutropenia.

a) P. aeruginosa bronchopulmonary infection are mostly seen in late stages of HIV infection. Most are community acquired and they recur or have chronic course despite proper antibiotic therapy.

b) P. aeruginosa bacteremia occur in children or adults with AIDS. It is either community or hospital acquired, the primary site of infection being lung, ear, upper respiratory tract or indwelling vascular catheters. The bacteremic infection are associated with skin manifestations, sepsis signs and may be fatal in children.

c) Paranasal sinus infection is a common complication in advanced HIV disease. It is community acquired and can recur or have chronic course. Multiple sinuses may be invoved.

d) Other AIDS associated Pseudomonas infection involve skin, soft tissue, urinary tract, bones. Malignant otitis externa not associated with diabetes mellitus also are reported.

BACTERIOCIN PRODUCTION:

The bacteriocin of Pseudomonas aeruginosa are called aeruginocin or more commonly pyocins named after the former species P. pyocyanaea. About 90% of the P. aeruginosa strains has the ability to produce pyocin which is called Pyocinogeny. There are four distinct types of pyocins.

1) R pyocin - Resembles the tails of contractile phages.

2) F pyocin - Resembles the tail of non contractile phages. It is flexuous, morphologically distinct rod shaped.

3) S pyocin - Low molecular weight, Trypsin sensitive.

4) S pyocin - Low molecular weight, Trypsin resistant.

There are a variety of individual pyocins within each category of pyocin which can be recognized based on their spectrum of activity against the different strains of P.aeruginosa. To their own pyocin ,the strains are immune but following attachment of pyocin to specific receptors upon the cell surface ,the sensitive cells are killed. More than one category of pyocin is produced by individual strains of P. aeruginosa and it also possess receptors for several different pyocins.

PYOCIN TYPING

In 1978, Govan described the standard procedure, cross streaking technique. In this method, the test strain is detected by inhibitory activity against 13 standard indicator strains of P.aeruginosa. But this method has the following disadvantages: the need to remove producer strain growth, the 72 hr time required for the result, the difficulty in typing mucoid strains of P.aeruginosa, the poor recognition of S Pyocin activity . Fyfe, Harris and Govan in 1984 developed a new revised spotting procedure which uses the

same typing pattern and retains the same 13 indicator strains. The procedure is described below.

1) The strains of P.aeruginosa to be typed are grown on Nutrient agar at 37 ° C overnight. Individual colonies of each strain to be tested are used in the preparation of $10^8 - 10^9$ organism in 1 ml of physiological saline which is sterile.

2) Multipoint inoculator is used (which incorporates 21 stainless steel pins of 2mm diameter set 16mm apart) to dispense 1µl volumes of the bacterial suspensions onto 13 plates arranged in a set (each plate with a diameter of 90 mm) which contains 10 ml Trptone Soya Agar. By this way, 20 test strains (one pin being a marker) are able to be typed simultaneously against each indicator strain. The plates are incubated after the spots are dried at 30 ° C for 6 hrs.

3) The filter paper discs (5cm, Whatman) are impregnated with chloroform upon which the plates are placed for about 15 minutes so that the vapour from the chloroform will kill the bacteria. The plate is exposed to air for about 15 minutes so as to eliminate the residual chloroform vapour.

4) Cultures of the indicator strains are prepared in Nutrient Broth without agitation at 37 ° C for about 4 hours to a population size of 10^7 organisms per ml.

5) Separate indicator strain is applied to each plate by adding about 0.1 ml of each bacterial indicator culture to 2.5 ml of molten semisolid agar(1% Peptone in 0.5% agar held at 45 º C); then it is poured as an overlay. The plates are incubated after the overlay is set at 37 º C for 8 hours.

6) The pyocin types of the test strains are determined similar to cross streaking method, based on the patterns of inhibition observed against the 13 indicator strains. But with the revised technique, the inhibition zone sizes are also taken into account for more detailed strain comparison. S pyocin activity determination is incorporated into the typing results similar to cross streaking method[22].

ANTIPSEUDOMONAL ANTIBIOTICS:

Carbapenems:

This belongs to a group of betalactum antibiotics which are obtained by the modification of Thienamycin, the parent antibiotic. From the bacteria Sreptomyces cattleya thienamycin is derived. The mechanism of action of carbapenems is by cell wall synthesis inhibition. They are bactericidal. They are not active against MRSA

and Enterococcus fecium. Bind to PBP1 and PBP2 of gram positive and gram negative bacteria, causing cell elongation and lysis[14].

IMIPENEM: It is highly active against P. aeruginosa in addition to Gram negative aerobes, Gram positive aerobes and many anaerobes. It is not active against Chlamydia and Mycoplasma species. It is administered parenterally as it is not absorbed orally. It is ineffective against intracellular organisms as it cannot penetrate intracellularly. The concentration of the active drug is more in plasma than the kidney because it is hydrolysed by dehydropeptidase in the kidney. So this drug is combined with cilastatin which inhinits dehydropeptidase and it doesnot have anti-microbial activity. Dosage: 500 mg i.m. 8 – 12 hrly or 1 - 2 g i.v. in 3 – 4 divided doses.

Meropenem: It is a dimethyl carbamoyl derivative of thienamycin. It does not require cilastatin as it is not susceptible to dipeptidase. It is similar to imipenem in its antibiotic spectrum[23].

Cephalosporins:

These are isolated from the fungus Cephalosporium acremonium.

Mechanism of action: It inhibits the cell wall synthesis and are bactericidal. There is increasing resistance to cephalosporins by betalactamases or by lack of bacterial permeability to the drug. There occurs cross resistance to other betalactum agents.

Third generation cephalosporins possess antipseudomonal activity. They have better CNS penetration than the first and second generation drugs. Fourth generation cephalosporins: Cefipime has similar properties as third generation cephalosporins but to some betalactamases it is more resistant than third generation cephalosporins.

Monobactums:

Aztreonam: It is a monobactum belonging to the class of betalactum antibiotics. Azreonam binds to PBP3(Penicillin Binding Protein 3) of gram negative aerobes,there by disrupting bacterial cell wall synthesis. They are active against Gram negative bacilli and inactive against Gram positive bacteria or anaerobes. They are resistant to the action of metallobetalactamases. It is given intravenously. The patients who are allergic to penicillin will tolerate aztreonam without any reaction.

Aminoglycosides:

Aminoglycoside is included as an empirical regimen for sepsis caused by P.aeruginosa[24]. Tobramycin: It is also available in ophthalmic solutions and ointments.

β-lactamase Inhibitors:

1. Clavulanic acid: It acts as a suicide inhibitor by forming an irreversible acyl enzyme complex with betalactamase, leading to loss of activity of the enzyme. Plasmid mediated TEM betalactamases present in Ceftazidime resistant strains of K. pneumoniae and E. coli are inactivated but inducible betalactamase (chromosomal class I) of Enterobacter and Pseudomonas species are not inhibited by this drug.

2.Sulbactam: It is an effective inhibitor of certain plasmid mediated and chromosomally mediated betalactamases of S. aureus and many Enterobacteriaceae[25] .

3.Tazobactam: It is suicidal betalactamase inhibitor and binds to bacterial PBP1 and PBP2. It remains inactive against the class 1 betalactamase of Enterobacter species, Pseudomonas species, S. maltophila and some Klebsiella species.

BETALACTAMASES:

Introduction-

Even before Penicillin was used in medical practice, the first betalactamase was identified in E. coli. Betalactamase production remains the most important contributing factor for betalactam resistance in gram negative bacteria[26].

Key Dates in Betalactamase emergence:

Year	Enzyme	Organism	Place
1944	Pencillinase	S. aureus	-
1963	TEM-1	E. coli	Athens
1974	SHV-1	E. coli	Switzerland
1978	OXA-10	P. aeruginosa	-
1988	Metallobetalactamase	P. aeruginosa	Japan
1991	OXA-14	P. aeruginosa	Turkey
1991	PER-1	P.aeruginosa, S. typhimurium	Turkey

METALLOBETALACTAMASE(MBL)

The first MBL was reported in the 1960s from Bacillus cereus and thereafter eighteen MBLs are observed in various Gram-negative bacteria. These MBLs production is mostly chromosome encoded which did not cause an obvious threat of spreading to other

bacteria. However the first plasmid mediated MBL was isolated in 1991 from Pseudomonas aeruginosa and was reported from Japan which was named as IMP-1, while VIM-1, an another type of acquired metallobetalactamase, was first reported in 1999 from Italy.

C**haracteristics of metallobetalactamase (MBL**):

1. Metallobetalactamase requires zinc for their catalytic activity.
2. Their activity is inhibited by metal chelators such as EDTA and THIOL compounds.
3. Metallobetalactamase in medical practice hydrolyse all betalactam antibiotics including Penicillins, Cephalosporins and Carbapenems with exception of Aztreonam (Monobactam).
4. MBL producing strains are not susceptible to serine betalactamase inhibitors (eg - clavulunate)[27].

Classification of MBL

1. According to Ambler Molecular classification - MBL belongs to class B. On the basis of their sequences, Class B is again divided into 3 subgroups which is class B1 to B3.

1. **Class B1**: These enzymes posses the zinc coordinating residues of one cysteine and three histidines and also will include the transferable MBLs.

2. **Class B2**: These enzymes will posess an asparginase at the first position instead of Histidine in the principal zinc-binding motif. They derive from the Serratia fonticola enzyme SFH- 1 and Aeromonas species.

3. **Class-B3**: MBL L1 is the sole occupant of this class, functionally represented as a tetramer[28].

2. **According to Bush-Jacoby-Medeirois (functional) Classification**: MBL belong to group 3.

On the basis of Imipenem and other betalactam antibiotic hydrolyzing capacity, Group-3 is again divided into 3 subgroups which include,

a. Group 3a - broad spectrum activity.

b. Group 3b – preferential activity towards Carbapenem.

c. Group 3c - hydrolyse Carbapenem poorly, as compared to other betalactam antibiotics[29].

3**. According to MBL gene location:**

a. Chromosomally encoded MBLs

b. Transferable MBLs (MBL gene as a part of integron structure).

4. **According to molecular classification:**

Transferable MBL is divided into 4 groups-

a. IMP(Imipenemase)Types

b. VIM(Verona Imipenemase)Types

c. GIM(German Imipenemase)Types

d. SPM(Sao Paulo Imipenemase)Types[30].

TRANSFERABLE MBLs:

Genetic Apparatus Of Transferable MBLs

Genes encoding IMP-, VIM-type as well as GIM-1 are found as gene cassettes in class 1 integrons[31]. The VIM and IMP type of MBLs are the most common[32,33]. Although IMP MBL genes are also found on class 3 integrons. Integrons are capable of procuring gene cassettes via a site-specific recombination event between two DNA sites, one in the integron and one in the gene cassette. Integrons consist of three regions: the 5′ conserved region, the 3′ conserved region, and a variable region. The 5′ region consists of the integrase gene, its adjacent recombination site, and a promoter, which facilitates expression of the procured gene cassettes in the variable region. The 3′ conserved region often consists of a partially deleted *qac* gene, fused

to a *sul* gene and correspondingly, confers resistance to antiseptics and sulfonamide, respectively.

Gene cassettes are small pieces of circular DNA, comprising a single gene together with a recombination site termed a 59-base element, approximately 1 kb in size. *bla*VIM genes from some European countries have been found with a truncated 59-base element and the gene cassettes are likely to be "fused". In most instances, this involves the MBL gene and an *aacA4* gene that encodes kanamycin, neomycin, amikacin and streptomycin resistance. Therefore, both aminoglycosides and betalactams will select clinical bacteria[27] harboring this fused gene cassette, further compromising these antibiotic regimens.

While gene cassettes carrying aminoglycoside and betalactam resistance genes can freely move from one integron to another, they cannot by themselves move from one organism to another and require the assistance of other genetic elements such as plasmids and transposons. The majority of MBL genes are found on plasmids usually between 120 and 180 kb. Not all MBL genes are associated with integrons or transposons. The genetic context of *bla*SPM-1 was shown to be unique, being adjacent to genes closely related to

Salmonella enteric serovar Typhimurium and not associated with an integron or transposon[28].

BIOCHEMISTRY OF MBLs

MBLs and serine betalactamase cleaves the amide bond of the betalactam ring and thus mediate resistance to betalactams; however, the way in which the two groups of enzymes achieve this differs considerably. MBLs possess a distinct set of amino acids that define the finite architecture of the active site which coordinates the zinc ions. The zinc ions in turn usually coordinate two water molecules necessary for hydrolysis. The principal zinc-binding motif is histidine-X- histidine-X-aspartic acid (HXHXD), which is common to most MBLs apart from the class B2 enzymes. Without exception, the preferred metal is zinc, and while most MBLs accommodate two zinc ions in their active site, the class B2 enzymes possess just a single zinc ion. The proposed mechanism of hydrolysis suggests that the active site orients and polarizes the betalactam bond to facilitate nucleophilic attack by zinc-bound water / hydroxides[34].

The MBL mechanism of hydrolysis is complex and varies from one MBL to another. MBLs may share less than 25%

amino acid identity with one another, they all share the unique αββα fold and their active site architecture is virtually superimposable.

It appears that most MBLs have a loop that is flexible and this is thought to facilitate binding and hydrolysis of the betalactam substrates. MBLs possess a wide plastic active-site groove and accordingly can accommodate most betalactam substrates, facilitating their very broad spectrum of activity. They are also impervious to the impeding effects of serine inhibitors such as clavulanic acid and sulbactum that are often treated as poor substrates. Interestingly, none of the MBLs hydrolyze aztreonam particularly well, and it has been speculated that it could be considered a therapeutic MBL inhibitor. Unfortunately, there are no standardized phenotypic methods available and the testing criteria are likely to depend on whether the gene is carried by P. aeruginosa or a member of the Enterobacteriaceae, i.e., the invincible level of resistance. For example, most Enterobacteriaceae and some Acinetobacter spp. Carrying MBL genes will appear 37 sensitive, with Imipenem MICs of between 1 and 2 μg/ml. Therefore, the implementation of a screening plate to detect MBLs, as has been advocated for extended-spectrum betalactamases, must take account of the genus of the bacterium, i.e., Pseudomonas

intrinsically have higher carbapenem MICs than Enterobacteriaceae. It is plausible that for screening Enterobacteriaceae.

The identification of some betalactamases has been aided by isoelectric focusing with the aid of counterstaining the gel with the chromogenic substrate nitrocefin to determine the enzyme's isoelectric point. This technique is based on the surface charge properties of these enzymes, which are neutralized at a certain pH. . For closely related enzymes e.g.,TEM and SHV, the isoelectric point represents a valuable tool in the identification process. However, MBLs, even the transferable types, differ considerably from one another, and thus, isoelectric focusing is not recommended as a tool to identify them, although it can provide useful information as to the isoelectric point of unknown MBLs by using EDTA inhibition (preincubated with the enzyme prior to electrophoresis or soaking the gel with EDTA after electrophoresis) as part of the isoelectric focusing process. Given the fact that all MBLs are affected by the removal of zinc from the active site, in principle, their detection should be straightforward, and studies have seized upon this principle and used a variety of inhibitor betalactam combinations to detect strains possessing these clinically important enzymes. However, MBLs

vary in their level of inhibition with certain compounds and also vary in their ability to confer resistance to ceftazidime or imipenem, two substrates commonly used in screening MBLs[35].

Risk factors for MBL colonization[36]

1. Prolonged hospital stay especially in ICU.
2. Irrational use of mulpitle antibiotics.
3. Patients on multiple invasive devices.
4. Presence of focal or generalized infection.

Phenotypic Methods of MBL detection[37]

Screening for MBL production was done in Meropenem resistant isolates by the following tests: Epsilometer or E test, Modified Hodge test, Combined disc test with meropenem as well as ceftazidime disc, Double Disc Synergy Test, EDTA Disc Potentiation using four cephalosporins.

The unique problem with MBLs is their unrivalled broad spectrum resistance profile. In addition, in many cases the MBL genes may be located on plasmids with genes encoding other antibiotic resistance determinants, i.e. aminoglycoside resistance genes. These MBL-positive strains are usually resistant to betalactams, aminoglycosides,

and fluoroquinolones. However, they usually remain susceptible to polymyxins.

No extended survey with a series of human infections with MBL-positive isolates has been performed to determine the optimal treatment. Thus, suitable therapy for treating those infections remains unknown. Using an animal model of pneumonia infection with a VIM-2- positive *P. aeruginosa* isolate, it was shown that aztreonam at a high dose reduced the bacterial load and may be a useful drug.

The only therapeutic alternative may be the therapeutic administration of polymyxins, which have recently been shown to be efficient for treating multidrug-resistant gram-negative bacilli. It has been claimed recently that polymyxins are not as toxic as previously thought. In any case, these molecules should not be used in monotherapy and rapid determination of MICs of aminoglycosides by MIC methods (not disk diffusion) may help to choose an aminoglycoside molecule that may have kept some activity. In addition, Rifampicin may be an interesting agent for treating multidrug resistant P. aeruginosa infections.

Clearly, in the absence of novel agents in the near future, the spread of MBL producers may lead to therapeutic dead ends. Early

detection may avoid spread of these multidrug-resistant isolates and may help maintain first and second line therapies.

INDIAN STUDIES on prevalence of P.aeruginosa infections and MBL prevalence and their detection :

Shampa Anupurba et al [38] (2006) isolated 301 strains of P. aeruginosa from 940 relevant clinical specimens accounting for 32% of various clinical specimens. She showed that P. aeruginosa were isolated higher in the age group of 16 - 40 years. Surgery ward has the highest prevalence rate (29.9%) with antimicrobial susceptibity for Cefaperazone/Sulbactum (74%) followed by Ciprofloxacin (58%) and Ceftazidime (54%) and 18% of isolates shows resistane to all the eight antibiotics.

Behera B et al[37] (2008) detected Metallobetalactamase in P. aeruginosa nosocomial isolates by four different phenotypic methods. 91 P. aeruginosa samples were subjected to antibiotic susceptibility testing by Vitek-2 and Disc diffusion assay. By three methods Imipenem resistance was determined (Disk diffusion, Vitek-2 and E-test). MBL production was detected by the following screening tests by combined disc test with Imipenem-EDTA, MBL E test with Imipenem-EDTA and EDTA disc potentiation test using four

Cephalosporins. Out of 63 isolates which were resistant to Imipenem, in 56 isolates MBL screening was done. Among 56 isolates, 36 were MBL positive by Double Disc synergy test and 48 were MBL positive by Combined disc test. E test was done in 30 isolates to confirm MBL production. All 30 isolates showed positivity by MBL E test. EDTA disc potentiation with four Cephalosporins was not useful for MBL detection.

Ami varaiya et al[39] (2008) found that out of 240 isolates, 60 (25%) were Carbapenem resistant and 50 (20.8%) were observed to be MBL producers. Among 50 MBL patients, 38 (76%) were Diabetic patients and 12 (24%) were found to be cancer patients.

Noyal et al[40] (2009) employed Modified Hodge test, EDTA disc synergy test to demonstrate MBL production in 32 Meropenem resistant P.aeruginosa. 16 (50%) were MBL producers by EDS test but only 9 positive for Carbapenemase by MHT and finally observed that EDTA disc synergy is more sensitive for detetion of MBL than MHT. They stated that both EDTA-Meropenem and EDTA Ceftazidime combination must be used in order to detect all MBL producers.

Attal Ro et al[36] (2010**) c**onducted study with total 140 P. aeruginosa strains isolated from various clinical samples. Antibiotic susceptibility testing with antipseudomonal was done as per CLSI guidelines. Imipenem resistant isolates were screened for carbapenem hydrolysis by the Hodge test and the modified Hodge test. MBL production was detected by DDST and Disk potentiation test. Out of 140 P. aeruginosa, 18(12.9%) were Imipenem resistant. Among these 16(88.8%) were Hodge test and modified Hodge test positive and all 16 were found to be MBL producers by the DDST and disk potentiation tests. He concluded that detection of MBL producing P. aeruginosa strains by the Disk potentiation test should be introduced in any clinical microbiology laboratory in order to aid in infection control.

Manoharan A et al[41] (2010) evaluated the Combined disc diffusion test (CDDT) method for screening and confirmation of metallobetalactamases with confirmatory E test and PCR among Carbapenem resistant P. aeruginosa isolates collected as a part of multicentric study (2005-2007). CDDT was done by using Imipenem, Meropenem and Ceftazidime with EDTA. MBL positives were confirmed by IMP + IMP-EDTA E test. They reported 42.6% to be

MBL producers among 61 isolates and 15 out of 20 MBL producer strains were positive strains for VIM type MBL. They reported CDDT using IMP+EDTA had highest sensitivity and specifity of 87.8% and 84% when compared to E test. CDDT using IMP+EDTA showed specifity 90.9% and sensitivity 93.3% when compared with PCR. They recommended routine use of IMP-EDTA CDDT test for screening of MBL producers among Pseudomonas aeruginosa.

D Bashir et al[42] conducted study with 283 P. aeruginosa isolates, 38 (13.42%) were resistant to Imipenem. 33 (11.6%) were found to be MBL producers by combined disk test and all of them showed reduction in MIC in the presence of Imipenem-EDTA in E test. The number of MBL positive isolates from ICU was statistically significant (p=0.027). The 46 hospital stay was significantly longer among patients infected with MBL producers than MBL non producers.

John et al[43] (2011) with the help of the following tests detected the presence of MBL production by Disc potentiation test, MHT, DDST and MIC by broth microdilution method. He observed MBL positivity in 92/331 isolates of Pseudomonas aeruginosa and stated that the DDST at 15mm distance was more reliable test than

CDT and MHT and the sensitivity was also good when zinc sulphate was dispensed as co-factor for MBL production .

Foreign studies on detection of MBL in P. aeruginosa-

Ibukun Abinu et al[44] (2007) in Nigeria studied occurrence of ESBL and MBL in P. aeruginosa between March and August 2006 isolated from various clinical specimens. ESBL and MBL were detected by using double disc synergy test and Imipenem EDTA combined disc test respectively. Results showed Carbapenems had highest activity followed by Ceftazidime (79.4%). Among 20 ceftazidime resistant isolates 9 were MBL producers, while 4 isolates showing resistance to carbapenems were ESBL producers. Amikacin was most potent among Aminoglycosides and they observed high level resitance to Fluoroquinolones.

Mohammed Ellalib et al[45] in Libya studied the detection and prevalence of metallobetalactamase in P. aeruginosa from August 2008 to August 2009 at Tripoli Medical Centre and Burn and Plastic Surgery Hospital among 312 specimens. The antibiotic resistance in this study is Amikacin (23.1%), Gentamicin (35.3%), Tobromycin (31.1%), Cefotaxime (51%), Ceftazidime (29.5%), Cefepime (28.2%), Ciprofloxacin (26.9%), Imipenem (13.8%), Meropenem (15.7%), Pipercillin

(39.1%), Carbenicillin (51.9%), Piperacillin/tazobactam (27.6%), Ticarcillin (53.8%), Aztreonam (22.8%), Polymyxin B (13.8%). Among the 312 isolates of *P. aeruginosa*, 42 ((13.8%)) were found resistant to Imipenem. Among them thirty four Imipenem resistant isolates were found to be MBL positive (10.9%). EDTA disc screen test is simple to perform and easy to interpret and can be introduced into the workflow of a clinical laboratory.

Eman et al[46] studied over a period of six months from December 2011 to May 2012 in Alhilla teaching hospital, to study the incidence of outbreak of MBL producing P. aeruginosa from various clinical specimens. Out of 247 clinical specimens 81 isolates were obtained . These isolates were subjected to susceptibility testing to anti pseudomonal drugs as per CLSI guidelines, 32 (40%) of isolates were carbapenem resistant P. aeruginosa (CRPA). They were further screened for production of MBL: by EDTA - Imipenem synergy method and Carbapenemase production: by modified Hodge test and using E. coli ATCC 25922 for control. All (CRPA) isolates were MBL producing isolates and carbapenemase producing isolates.

MATERIALS & METHODS

MATERIALS AND METHODS

Place of study

The present study was conducted in Coimbatore Medical College and Hospital, Coimbatore.

Study Period

The study period was conducted for a period of one year from August of 2013 to July of 2014.

Type of Study

It is a prospective study.

Ethical consideration

Before the study period, the Ethical and Research clearance was obtained from Ethical Committee of Coimbatore Medical College and Hospital, Coimbatore.

Sample

In the present study 211 Pseudomonas aeruginosa were isolated from different clinical samples like pus, urine, blood, aural swab, wound swab, catheter tip and sputum.

Inclusion Criteria

1) Out of all the organisms identified as Pseudomonas aeruginosa the consecutive samples are included in the study.

2) Only the samples which show intermediate or resistance to meropenem are included in detection of metallobetalactamase by phenotypic methods.

Exclusion Criteria

1) All other clinical isolates excluding Pseudomonas aeruginosa isolated.

PROCESSING OF SAMPLE:

All samples were collected under strict aseptic precautions under standard procedures and processed under standard procedures.

1) Direct Gram stain:

Direct smears with gram stain was done to look for the presence of pus cells and presence of bacteria. Gram stain shows plenty of pus cells with Gram negative bacilli.

2) For all the samples except blood, the samples were inoculated into the MacConkey agar and Blood agar and incubated for 18 – 24 hours at 37 º C.

3) For blood and catheter tip culture, BHI broth is used in which the samples were inoculated and they are kept in incubator at 37 º C for 48 hours. The broth was examined for turbidity regularly and subculture was done on Blood Agar plate as well as MacConkey Agar at regular intervals. Any growth was further processed for identification.

4) The non lactose fermenting colonies on MacConkey agar which was oxidase positive were being further processed for identification.

BIOCHEMICAL REACTIONS:

The non lactose colonies are subjected to the following set of tests at first.

1. Gram Stain
2. Motility.
3. Catalase Test.
4. Oxidase Tests.

If the organism is a gram negative bacilli, motile, catalase test positive, Oxidase test positive, then it is subjected to the following group of reactions.

S.No	Biochemical tests	Results
1	Indole test.	Negative
2	Simmon's Citrate Utilisation Test.	Positive
3	Christensen's Urease hydrolysis Test.	Negative
4	Kligler's Iron Agar Test.	Alkaline slant / No change
5	Arginine Dihydrolase Test.	Positive
6	Nitrate reduction tests.	Positive
7	Glucose Oxidation tests.	Oxidative utilization.

CATALASE TEST:

Principle:

The principle of this test is to identify the presence of catalase. This enzyme catalyses the reaction in which oxygen is released from Hydrogen peroxide.

Procedure:

One or two colonies to be tested is picked up by the glass rod which must be sterile. It is inserted into 3% hydrogen peroxide kept in a sterile glass tube.

Interpretation:

Presence of effervescence or gas bubbles formation immediately or within seconds will indicate the positive catalase test and no bubble formation immediately will be reported as negative catalase test[47].

OXIDASE TEST:

Principle:

This test identifies the presence of Cytochrome oxidase enzyme in the bacteria. It catalyses the reaction in which oxygen oxidizes the reduced cytochrome enzyme.

Procedure:

Filter paper strips soaked in the oxidase reagent (1% tetramethyl para phenylene diamine hydrochloride) is placed in a petridish and the colony to be tested is smeared on the strips using sterile glass rod.

Interpretation:

Development of purple colour within 10 sec is interpreted as positive.

NITRATE TEST:

Principle:

This test detects the nitrate reductase enzyme present in the bacteria which reduces nitrate to nitrite.

Procedure:

Organism is grown in 5ml of nitrate broth for 24 to 48 hours. Equal volumes of A and B are mixed just before use. 0.1 ml of the reagent mixture is added to the culture.

Interpretation:

Development of red colour within few minutes is considered positive.

Indole test

Principle:

This test identifies the presence of enzyme tryptophanase in the bacteria. This enzyme degrades the tryptophan to Indole.

Interpretation:

A pink colour ring development indicates a positive test.

Urease test:

Principle:

By this test we identify the presence of urease enzyme. This urease can split urea to ammonia and Carbon dioxide .

Procedure:

Test organism is inoculated on to slope of Christensen's urease medium and incubated for 24 – 48 hours at 37 ° C .

Interpretation:

Development of pink colour in the slope is interpreted as positive test.

Citrate test

Principle:

In this test we are testing the ability of organism to use citrate as the sole source of carbon .

Procedure:

Test organism is inoculated into slope of Simmon's citrate medium and incubated at 37 ° C for 24 – 48 hours.

Interpretation:

Development of blue colour in the medium is interpreted as positive.

Kligler Iron agar test (KIA agar):

KIA agar medium contains 10 parts lactose, 1 part glucose and peptone. Phenol red and Ferrous sulphate serve as indicators of acidification and hydrogen sulphide respectively. With straight inoculation wire, touch the top of well isolated colony. Inoculate KIA by first stabbing through the centre of the medium to the bottom of the tube and then streaking the surface of agar slant. Incubate the tube at 37 º C in ambient air for 18 – 24 hours.

Results:

Alkaline slant / no change in butt (K / NC): Glucose and lactose non utilisers.

Alkaline slant / acid butt (K / A): Glucose fermentation only.

Acid slant / acid butt (A / A): Glucose and lactose fermenter.

A black precipitate in the butt indicates production of ferrous sulphate and hydrogen sulphide gas. Bubbles or cracks in the tube indicate the production of CO_2 or H_2S.

Arginine dihydrolase test:

Dissolve the Arginine dihydrolase medium in water and adjust pH to 6.0. This is the basal medium used as control and to this add 1% L-Arginine dihydrochloride to make it test medium.

The medium is purple in colour as the indicator is bromocresol purple. Both the test tubes are covered with mineral oil to give an anaerobic environment. After adding the turbid peptone water of the test organism it is incubated at 37 º C for 48 hours .

Results:

The medium turns to yellow in between and then reverts back to purple colour. This indicates that this test is positive.

SUGAR FERMENTATION TESTS:

Principle:

To demonstrate the ability of the organisms to ferment specific sugars incorporated within the medium producing acid and gas.

Procedure:

The media contains peptone water with 1% sugars (Glucose, Lactose, Sucrose, Maltose, Mannitol, etc.) along with an indicator (Bromothymol blue) and Durham's tube. To the sugar media, 0.5 ml of the 0.5 McFarland standard of the organism to be tested is inoculated into the sugar media and it is incubated for 16 - 18 hours at 37 º C.

Interpretation:

The blue colour of the medium turns yellow with acid production by bacteria and production of gas is indicated with gas bubbles formation in the Durham's tube.

ANTIOBIOTIC SUSCEPTIBILITY TESTING:

The antibiotic susceptibility testing for P. aeruginosa isolates was done by Kirby Bauer Disc Diffusion method as given in CLSI guidelines. In the present study susceptibility was tested against following antibiotics obtained commercially from Hi-media laboratories Limited, Mumbai. For control we used P. aeruginosa ATCC 27853 strain. The diameter of the zone was measured and interpreted as sensitive, intermediate, resistance according to the CLSI guidelines[48].

Antibiotic discs	**Concentration in µg**	**Sensitive zone in mm**	**Intermediate zone in mm**	**Resistance zone in mm**
Amikacin(Ak)	30	≥17	15-16	≤14
Gentamicin(G)	10	≥15	13-14	≤12
Ciprofloxacin(Cip)	5	≥21	16-20	≤15
Ofloxacin(Of)	5	≥16	13-15	≤12
Ceftazidime(Caz)	30	≥18	15-17	≤14

Cefaperazone Sulbactum(Cfs)	75/30	27 – 33		
Cefipime(Cpm)	30	≥18	15-17	≤14
Tobramycin(Tob)	10	≥15	13-14	≤12
Piperacillin Tazobactum(Pit)	100/10	≥21	15 - 20	≤14
Meropenem(Mrp)	10	≥19	16-18	≤15
Aztreonam(Azm)	30	≥22	16-21	≤15

The Meropenem resistant isolates are subjected to the following four different phenotypic tests to detect thr production of metallobetalactamases.

PHENOTYPIC METHODS TO DETECT METALLOBETALACTAMASE:

1) MODIFIED HODGE TEST:

The Modified Hodge test (MHT) was originally described by CDC (Centre for Disease Control, Atlanta) for Carbapenemases detection in Enterobacteriaceae. But in this study I have included this test for Metallobetalactamase detection in P. aeruginosa.

Procedure:

In the first step, 0.5 McFarland's standard of E.coli ATCC 25922 is prepared in 5 ml of peptone water. Then 1:10 dilution is obtained by adding 0.5 ml of the 0.5 McFarland's standard to 4.5 ml of peptone water. This is used to make a lawn culture on a Mueller Hinton agar plate and allowed to dry for about 3 -5 minutes. A 10µg Meropenem disc was placed in the centre of the plate. Then one particular P. aeruginosa isolate were streaked from edge of the disc to the periphery of the plate in a straight line using a 0.5 mm loop in four different directions like a cross. In this test four different isolates can be tested in a single plate also.

Interpretation of Results:

The plate is examined after incubating for 16–24 hours at 37 º C, for a clover leaf like indentation which is seen at the intersection of the organism which is tested and the E. coli 25922. It is also seen within the inhibition zone of the meropenem susceptibility disc.

MHT Positive test has the indentation of the E.coli 25922 in the shape of a clover leaf. It grows along the test organism growth streak

and within the disc diffusion zone. A positive MHT indicates carbapenemase production by that particular isolate.

MHT Negative test has no growth of the E.coli 25922 within the disc diffusion as well as along the test organism growth streak. A negative MHT indicates carbapenemase is not produced by that isolate.

2) MEROPENEM –EDTA COMBINED DISC TEST (CDT):

We performed the Meropenem EDTA combined disc test as described in the study by **Yong et al** [49] . The 0.5 M EDTA solution was prepared by dissolving 18.61 g of EDTA in 100 ml of distilled water and pH was adjusted by using sodium hydroxide to 8. The prepared solution was sterilized by autoclaving. The organisms to be tested were inoculated on to Mueller Hinton agar plates according to the CLSI guidelines. Then we placed two meropenem discs (Hi Media) of 10µg concentration on the plate and appropriately 10 µL of EDTA solution were added to one meropenem disc with the help of a micropipette to obtain the desired concentration of 750 µg. The plates were incubated for 16 to 18 hours at 37 º C and the inhibition zones of the Meropenem with EDTA disc and Meropenem were compared. In the combined disc test, if there is ≥ 7 mm increase in inhibition zone size around Meropenem with EDTA disc than the Meropenem

disc alone, then the test is positive. It indicates that the test organism produces metallobetalactamase enzymes.

3) MEROPENEM – EDTA DOUBLE DISC SYNERGY TEST (DDST):

The double disc synergy test of Meropenem and EDTA was done as described in the study by **Lee et al**[50] . The organism to be tested were inoculated on to the Mueller Hinton agar plate as per the CLSI guidelines. A 10 µg Meropenem disc was placed on the MHA plate at a distance of 20 mm centre to centre from the blank disc which contains 10 µL of 0.5 M EDTA to get the required 750 µg concentration. The MHA plate is then incubated at 37 º C for a duration of 16 to 18 hours. If there was enhancement in the inhibition zone of > 5 mm in the area between Meropenem disc and the EDTA disc, then the test is positive which identifies the test organism as a metallobetalactamase producer.

4) Epsilometer or E TEST: Meropenem with & without EDTA Ezy MIC Strips:

(Meropenem + EDTA: 1-64 mcg / ml)
(Meropenem : 4- 256 mcg / ml)

It is a unique Phenotypic MBL detection test which contains a strip which is coated with mixture of Meropenem + EDTA and Meropenem on a single strip in a concentration gradient

manner. It is procured from high media laboratories. The upper half has Meropenem + EDTA with highest concentration tapering downwards, whereas lower half is similarly coated with Meropenem in a concentration gradient in reverse direction.

Test Procedure

1. At first 0.5 Mcfarland standard of the P. aeruginosa isolate to be tested is prepared by inoculating two to three colonies onto peptone water and incubating it for 2 - 3 hours at 37 º C.

2. Then a cotton swab which is sterile attached on a wooden stick is dipped into the 0.5 McFarland standard inoculums. The swab which is soaked is firmly rotated against the inside wall in the upper part of the tube to express excess fluid. With the help of the swab the whole plate on its agar surface is streaked three times with the swab, rotating at an angle of 60 º C in between each streaking.

3. Ezy MIC strip is removed from the container from cold and it is kept at room temperature for 15 minutes before opening. Then it is applied on to the centre of the MHA plate with the help of a wooden stick.

4. The MHA plate is incubated at 37 º C for 18 - 24 hours and then the following values are observed.

a. MIC of Meropenem

b. Concentration of MRP + EDTA.

c. Ratio of MRP / MRP +EDTA

Interpretation of E Test:

MBL POSITIVE STRAIN:	$\frac{MRP}{MRP+EDTA} => 8$ $\frac{MRP}{MRP+EDTA} = \frac{>256}{<64}$ $\frac{MRP}{MRP+EDTA} = \frac{>256}{<1}$	When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP+EDTA) is more than to 8 or If zone is observed on the side coated with Meropenem+EDTA & no zone is observed on the opposite the side coated with Meropenem, interpret the culture as MBL positive.
MBL NEGATIVE STRAIN:	$\frac{MRP}{MRP+EDTA} \leq 8$	When the ratio of the value obtained for Meropenem (MRP): the value of Meropenem+EDTA (MRP+EDTA) is less than or equal to 8
MBL (NON DETERMINATIVE)	$\frac{MRP}{MRP+EDTA} = \frac{>256}{>64}$ $\frac{MRP}{MRP+EDTA} = \frac{<256}{<4}$	When no zone of inhibition is obtained on either side. In such cases resistance may be due to mechanisms other than MBL production. These have to be further investigated before reporting. or If the zones obtained are below the lowest concentration on both the sides, the strain has to be tested with concentrations below the lowest concentration on the strips before reaching to any conclusion.

QUALITY CONTROL:

Quality control of Ezy MIC Strips is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.

Organism	**Medium used**	**Incubation**	**Standard**
Pseudomonas aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs	When the ratio of the value obtained for Meropenem (MRP) : the value of Meropenem + EDTA (MRP + EDTA) is less than or equal to 8.

Fig. i: MacConkey Agar Plate showing Pseudomonas aeruginosa

Fig. ii: Blood Agar Plate showing Pseudomonas aeruginosa

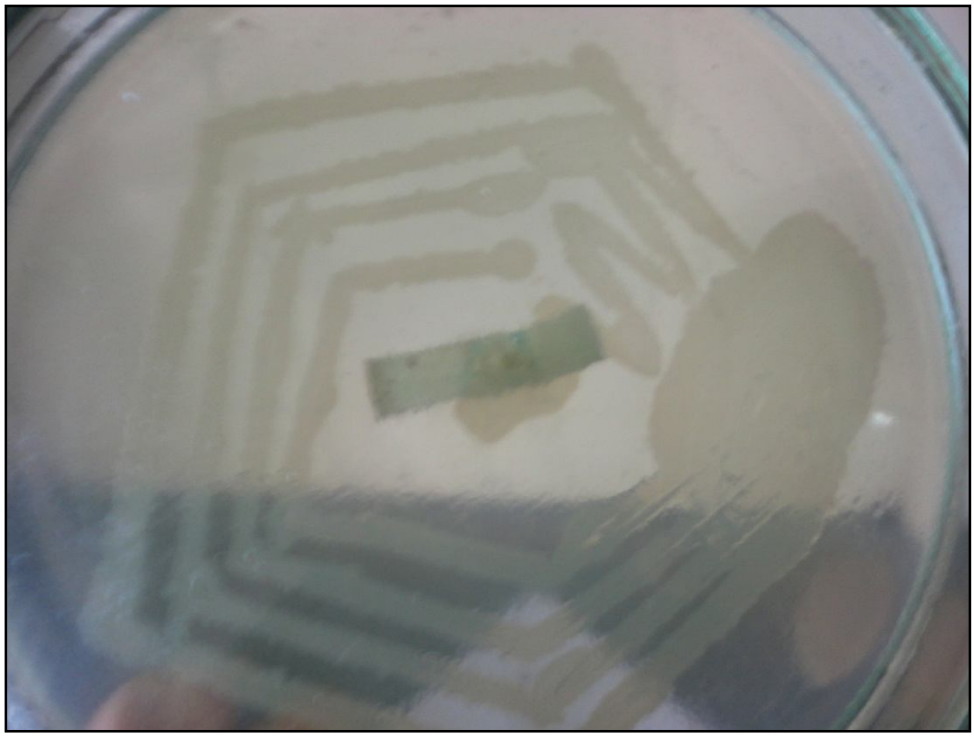


Fig. iii: Nutrient Agar Plate showing Pseudomonas aeruginosa

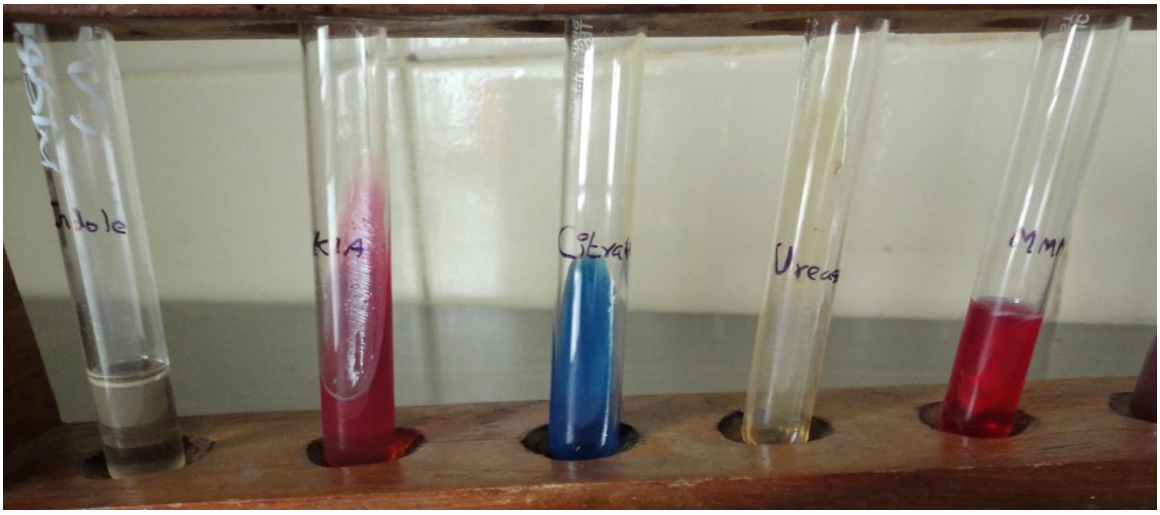


Fig. iv: Biochemical reactions of Pseudomonas aeroginosa

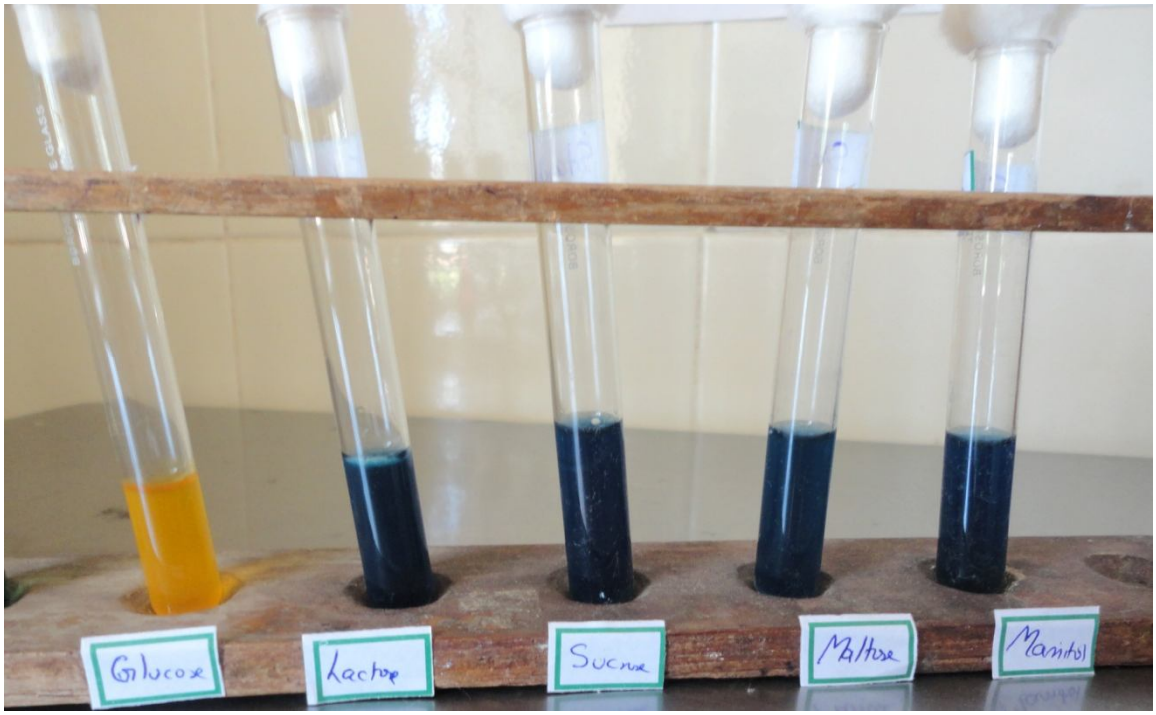


Fig. v: Sugar Fermentation Test showing Glucose Oxidation

Fig. vi: Moeller's Decarboxylase (LAO) Test

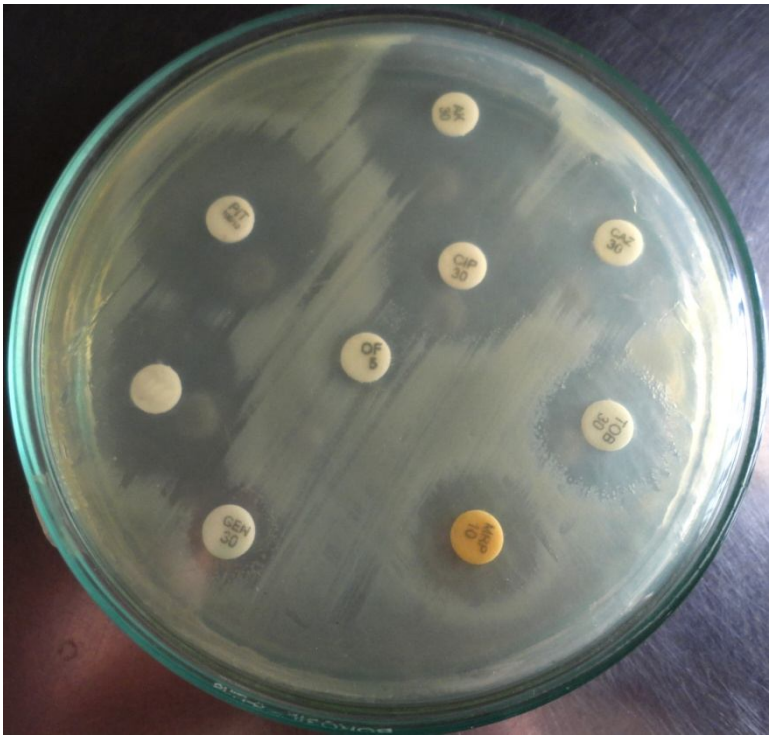


Fig. vii: AST Plate showing Meropenem Resistance

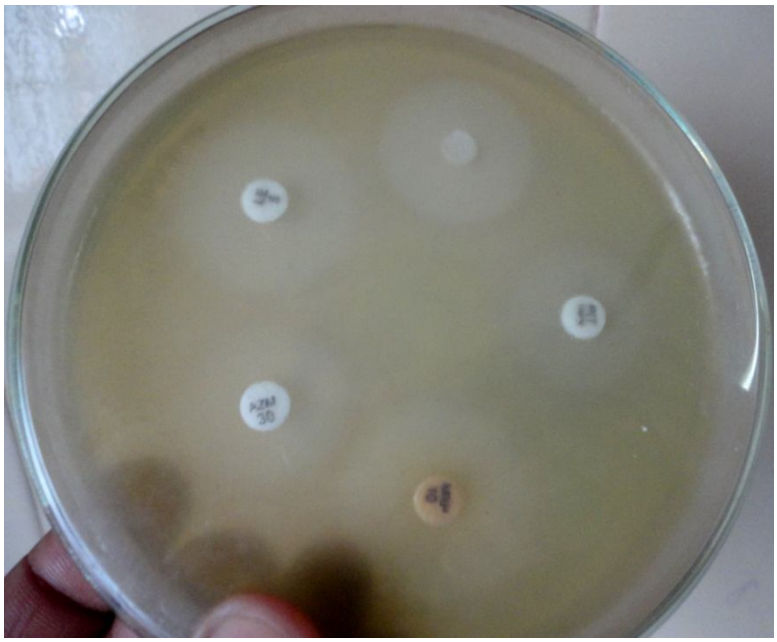


Fig. viii: AST Plate showing Meropenem Sensitivity

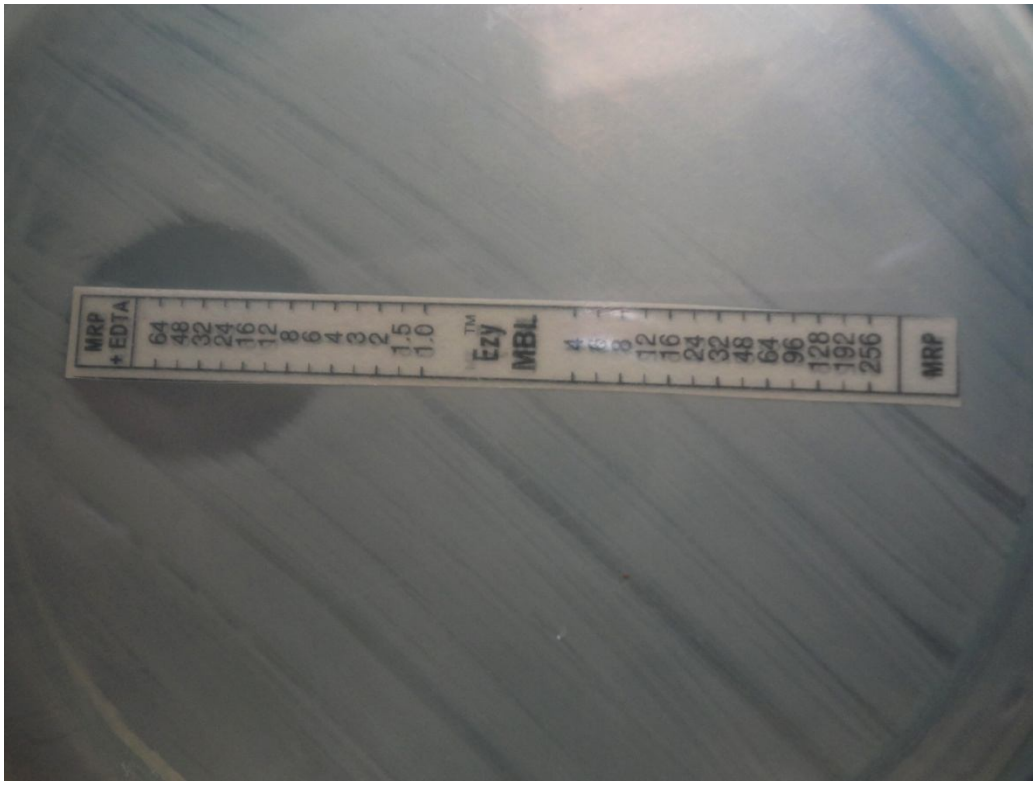


Fig. ix: E Test Positive

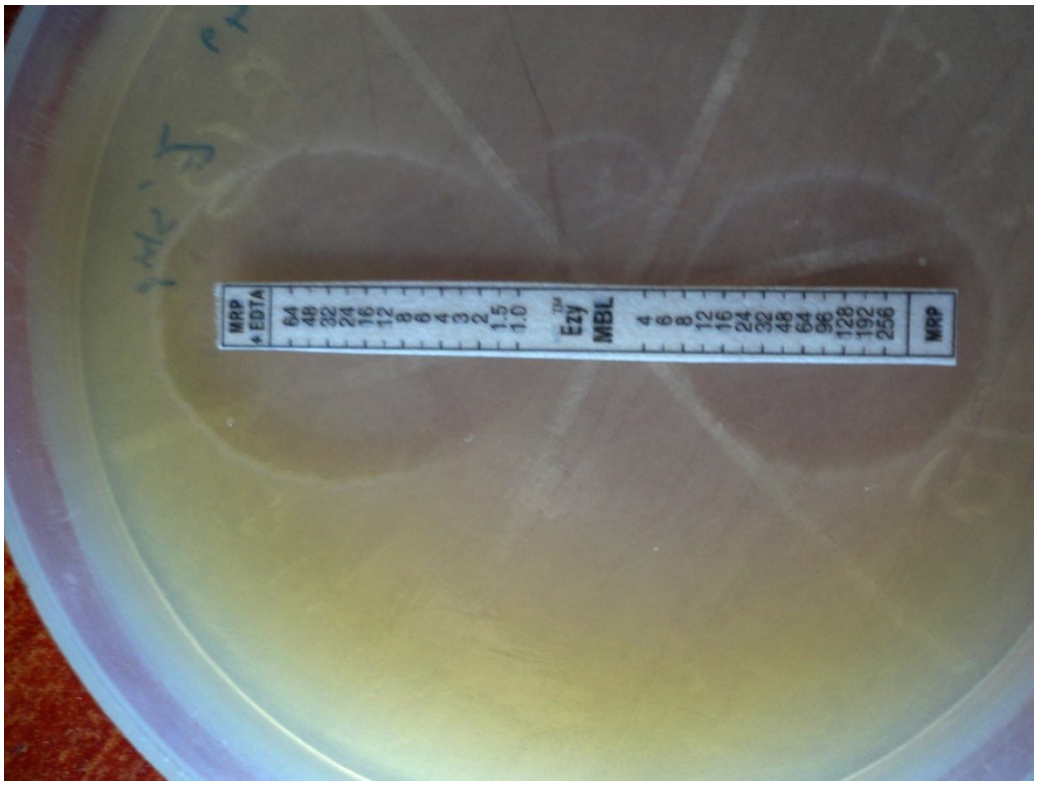
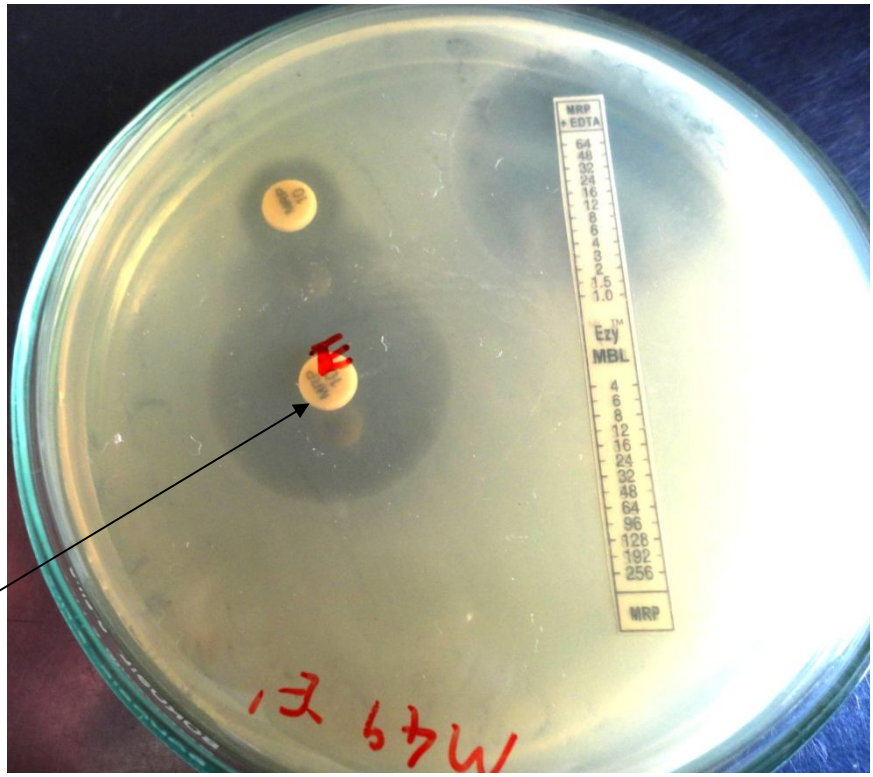
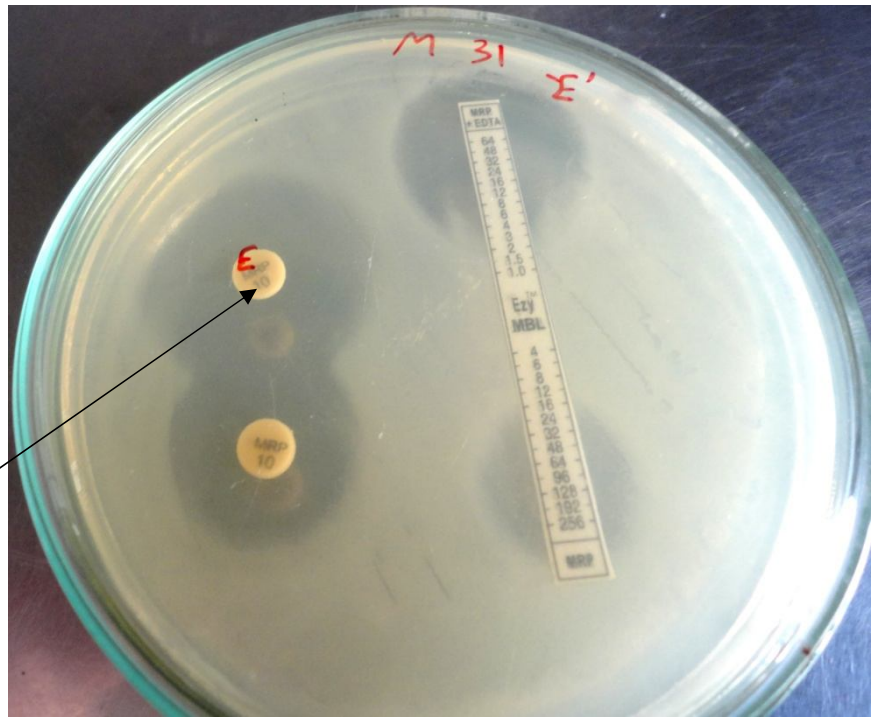


Fig. x: E Test Negative



Meropenem with EDTA

Fig. xi: Combined Disc Test Positive



Meropenem with EDTA

Fig. xii: Combined Disc Test Negative

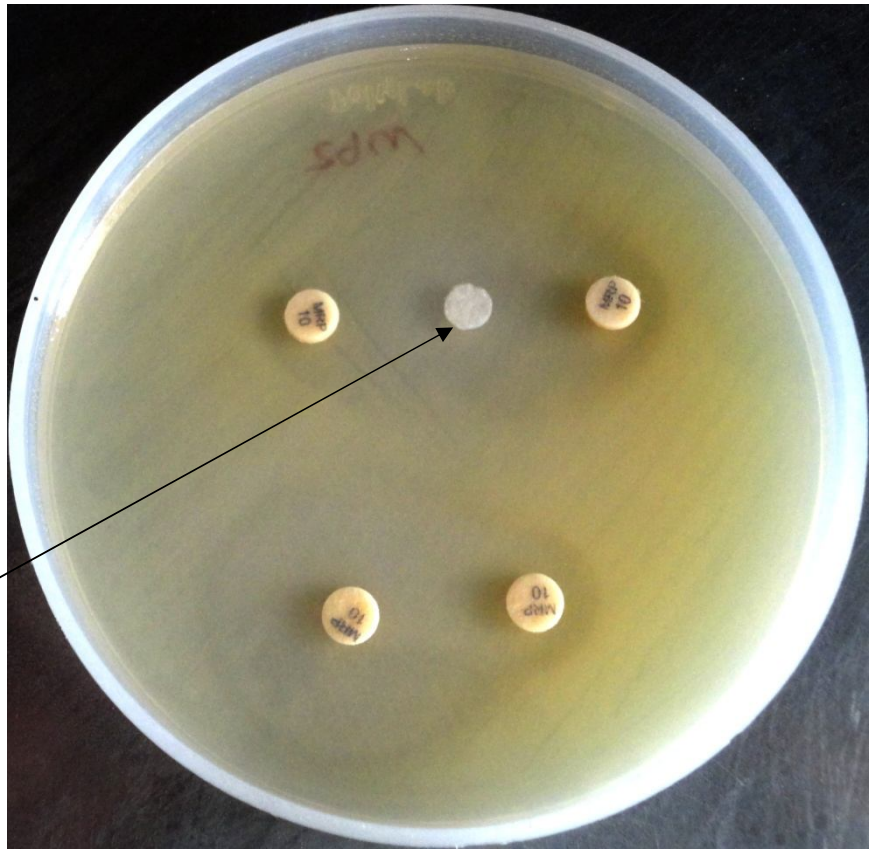


Fig. xiii: Double Disc Synergy Test Positive

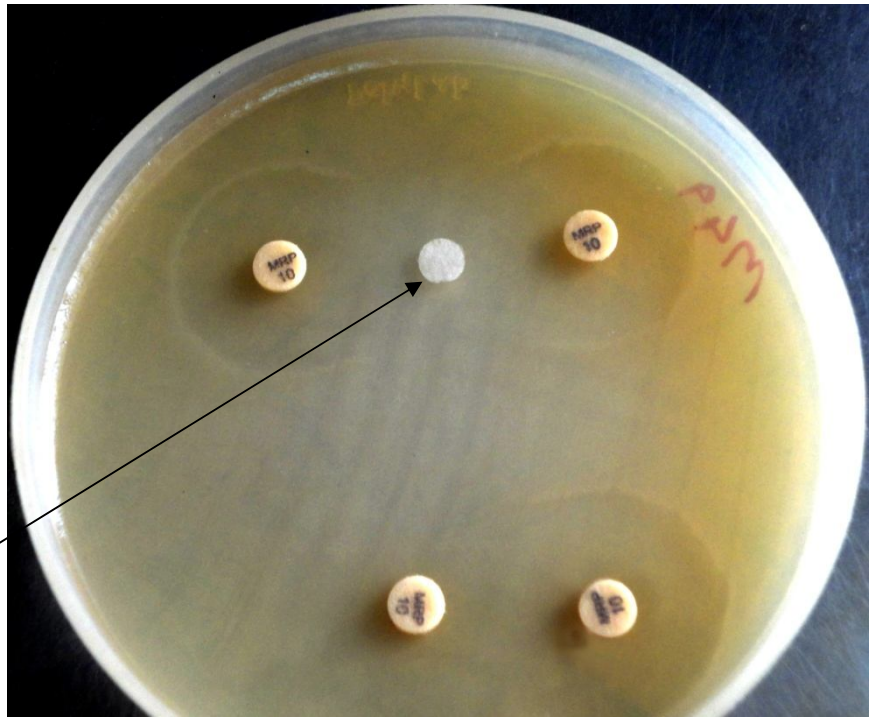


Fig. xiv: Double Disc Synergy Test Negative

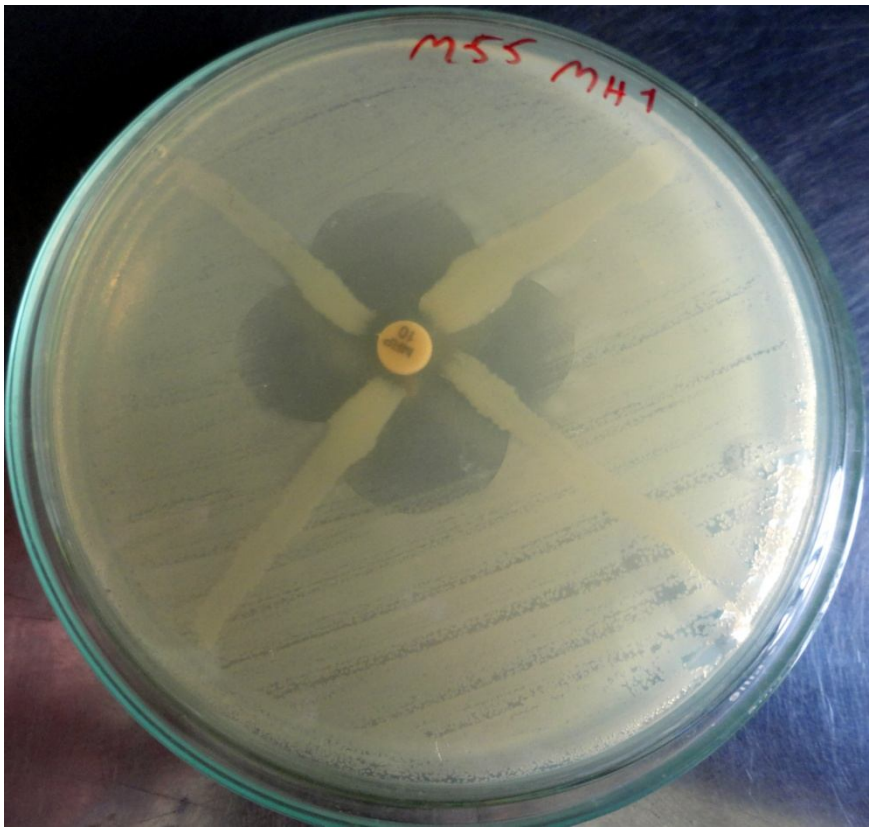


Fig. xv: Modified Hodge Test Positive

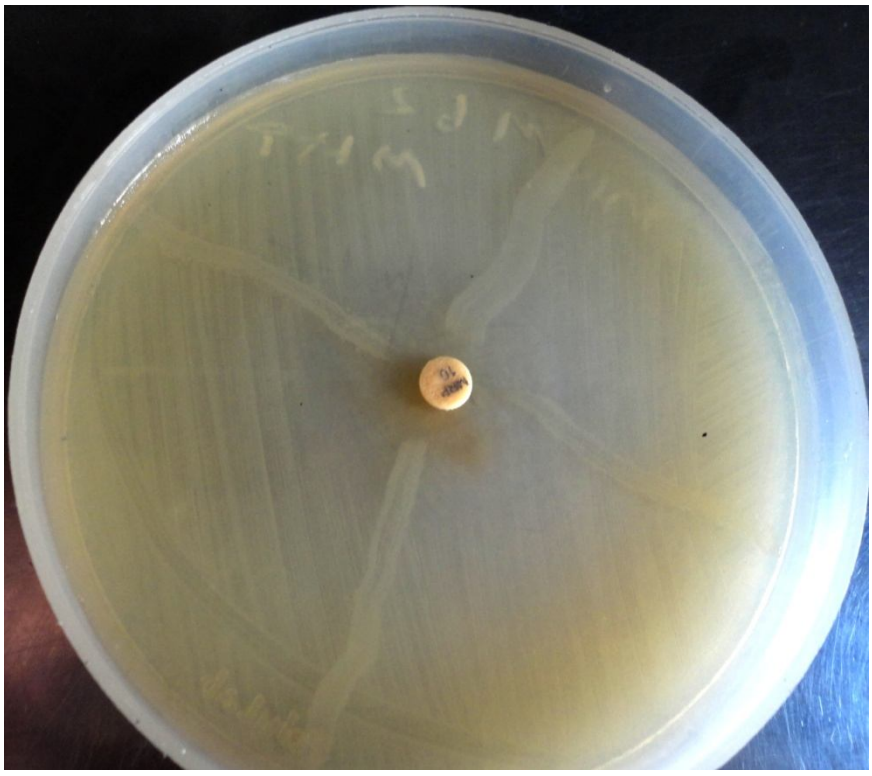


Fig. xvi: Modified Hodge Test Negative

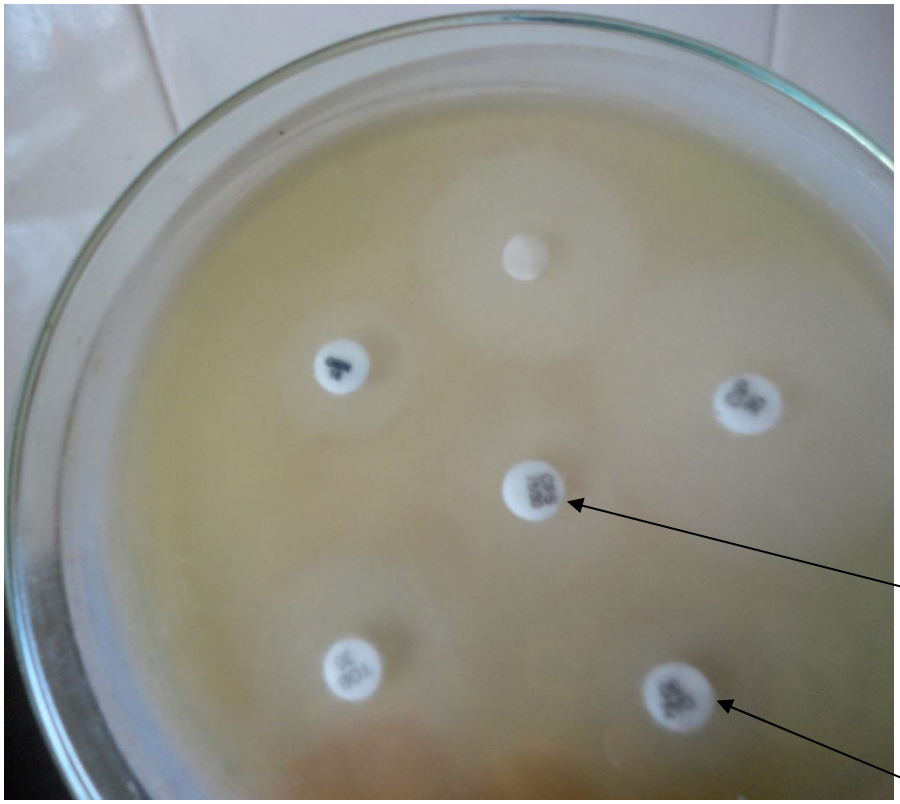


Fig. xvii: AST Plate showing Ceftazidime and Cefaperazone sulbactum resistance

RESULTS

RESULTS

In the present study 211 Pseudomonas aeruginosa isolates from various clinical samples like pus, urine, wound swabs, sputum, burns were taken from 211 patients during August 2013 to July 2014 at Coimbatore Medical College and Hospital, Coimbatore. These isolates were studied for detection of Metallobetalacamase production and their antibiogram.

The antibiotic susceptibility of P. aeruginosa isolates indicated the following rates of sensitivity: Amikacin (67.77%), Gentamicin (63.98%), Ciprofloxacin (53.55%), Ceftazidime (28.91%), Cefoperazone sulbactum (64.93%), Cefipime (70.14%), Piperacillin + tazobactum (81.04%), Tobramycin (64.93%), Meropenem (92.42%), Aztreonam (76.78%) which is shown in table – 1. Among the P. aeruginosa isolates from urine Ofloxacin showed 76.2%, Norfloxacin showed 28.57% sensitivity and Nitrofurantoin were 80.95% resistant.

In this study , out of 211 isolates, 16 (7.58%) were found to be Meropenem resistant by disc diffusion method as seen in table - 2 .

The MIC of Meropenem among different MBL producing P. aeruginosa isolates are in the range of 24 – 256 µg as observed in table - 3.

The percentage of MBL detected among MRP resistant P. aeruginosa isolates are 93.75 % by MRP / MRP+EDTA E test, 81.25 % by Double Disc Synergy Test, 81.25 % by Combined Disc Test, 75 % by Modified Hodge Test . This is visualised in Table - 4.

In table - 5, the 2 × 2 table shows the comparison of the combined disc method with E test . Test validity parameters were calculated from the table. The sensitivity, positive predictive value and accuracy of Combined Disc Test were found to be 86.67 %, 100 % and 87.5 % respectively.

In table - 6, the 2 × 2 table shows the comparison of the double disc synergy method with E test. Test validity parameters were calculated from the table. The sensitivity, positive predictive value and accuracy of Double Disc Synergy Test were found to be 86.67 %, 100 % and 87.5 % respectively.

In table - 7, the 2 × 2 table shows the comparison of the Modified Hodge test with E test. Test validity parameters were calculated from the table. The sensitivity, positive predictive value and accuracy of Modified Hodge Test were found to be 80 %, 100 % and 97.5 % respectively.

In table - 8, the 2 × 2 table shows the comparison of the E test with MBL producers and Non MBL producers. The sensitivity, specificity, positive predictive value, Negative predictive value and accuracy of E Test were found to be 100 %, 100 %, 100 %, 100 % and 100 % respectively.

As from the Table - 9 the Metallobetalactamase prevalence among total P. aeruginosa isolates in our study is 7.11%. The Metallobetalactamase prevalence among Meropenem Resistant isolates in our study is 95.5 %. This is shown in table - 10.

In our study, from the table - 11 we observed that the cases are distributed in the following age groups: 0 - 10 years(14.22 %) 11 – 20 years(6.64 %) , 21 – 30 years (12.32 %) , 31 – 40 years(13.27 %) 41 – 50 years(14.22 %), 51 – 60 years (15.64 %) and above 60 years (23.69 %) . MBL producing isolates are seen in the age group of > 60 years.

The sex wise distribution of cases according to table - 12 are: males - 143(67.77%) amd females - 68 (32.23%). The male : female sex ratio is 2.1 : 1 . Among MBL isolates, 9 (60%) are males and 6 (40%) are females. The male female sex ratio is 3 : 2 .

Males are more commonly distributed among the above 40 years age group about 54.44%; while females are 51.46% among among the above 40 years age group according to table - 13.

Among the isolates 199 (94.3%) were from inpatients and 2 (5.69%) were from outpatient department. Similarly in MBL producers 93.33% were isolated from inpatient department (IPD). Only 6.67 % were isolated from outpatient department. This is seen in table - 14.

In our study, the distribution of isolates from different wards as observed in table 15 are from Surgery 66 (31.28%), 48 (22.75%) from Orthopaedics, 35 (16.59%) from General Medicine, 214(11.37%) from Paediatrics and medicine ICUs, 11 (5.21%) from OPD, Others from Nephrology, Urology, ENT, Burns and Skin wards. Among MBL producers 6(40%) were from surgery, 4 (26.67%) were from Orthopaedics, 2 (13.33%) from burns ward, 1 (6.67%) each from OG and Urology ward.

In this study, P. aeruginosa were isolated from the clinical samples as follows: 131 (62.09%) isolates among 211 were from pus, 30 (14.22%) from sputum, 21 (9.95%) from urine, 18 (8.54%) from Blood, 5 (2.37%) from Ear discharge, 4(1.9%) from Burns ward and 1 (0.47%) each from Catheter Tip and ET Tube. Out of 6 MBL producers 11 (73.33%) were from pus, 2 (13.33%) from burn ward and 1 (6.67%) each from Urine and catheter tip. These are shown in Table - 16.

In the present study, Pseudomonas aeruginosa isolates were obtained from cases of Cellulitis [19.9%], followed by traumatic wound infections [18.47%], cases of diabetic foot ulcers [16.59%], Pneumonia [14.22%], Urinary tract infections [8.54%], Fever cases [5.69%], Septicemia [5.21%], Necrotising Fascitis [4.27%], Post operative wound infections [2.84%], CSOM [2.37%], Burns cases [1.9%] as seen in table - 17. Among MBL producers the isolates were obtained from infections of Trauma wound [26.67%], Cellulitis [20%], Diabetic foot ulcer [20%], Burn wound [13.325], urinary tract infections, Post operative wound infection and septicemia each constitute 6.67% .

The antibiotic resistance among MBL isolates are Amikacin (66.67%), Gentamicin (73.33%), Ciprofloxacin (73.33%),

Ceftazidime (100%), Cefaperazone sulbactum (100%), Cefipime (100%), Piperacillin + tazobactum (60%), Tobramycin (100%), Meropenem (100%), Aztreonam (86.67%) which is shown in table - 18. The urine isolates are 100 % resistant to Ofloxacin, Norfloxacin and Nitrofurantoin.

TABLE - 1: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES :

ANTIBIOTICS	P. aeruginosa Isolates (n = 211)	
	SENSITIVITY	RESISTANCE
AMIKACIN	143(67.77%)	68(32.23%)
GENTAMICIN	135(63.98%)	76(36.02%)
TOBRAMYCIN	137(64.93%)	74(35.07%)
CIPROFLOXACIN	113(53.55%)	98(46.45%)
CEFTAZIDIME	61(28.91%)	150(71.09%)
CEFAPERAZONE SULBACTUM	137(64.93%)	74(35.07%)
CEFIPIME	148(70.14%)	63(29.86%)
MEROPENEM	195(92.42%)	16(7.58%)
AZTREONAM	162(76.78%)	49(23.22%)
PIPERACILLIN TAZOBACTUM	171(81.04%)	40(18.96%)
AMONG URINE ISOLATES (n = 21)		
OFLOXACIN	16(76.2%)	5(23.8%)
NORFLOXACIN	6(28.57%)	15(71.43%)
NITROFURANTOIN	4(19.05%)	17(80.95%)

TABLE - 2 PREVALENCE OF MEROPENEM RESISTANCE AMONG ISOLATES

TOTAL NO OF CASES	NO OF MRP RESISTANT ISOLATES	% OF MRP
211	16	7.58%

TABLE - 3 MIC OF MEROPENEM RESISTANT ISOLATES

MRP RESISTANT ISOLATE	MIC OF MRP	MIC OF MRP + EDTA	RATIO OF MRP/ MRP+EDTA	MBL PRODUCED
1	>256	4	>8	YES
2	64	1	>8	YES
3	24	<1	>8	YES
4	256	2	>8	YES
5	96	1.5	>8	YES
6	256	2	>8	YES
7	128	4	>8	YES
8	96	1.5	>8	YES
9	>256	16	>8	YES
10	64	16	< 8	NO
11	24	<1	>8	YES
12	>256	2	>8	YES
13	128	4	>8	YES
14	48	<1	>8	YES
15	>256	16	>8	YES
16	128	1.5	>8	YES

TABLE - 4 DETECTION OF MBL BY FOUR DIFFERENT PHENOTYPIC METHODS

TOTAL MRP RES ISOLATES	BY E TEST	BY MHT	BY CDT	BY DDST
16	15/16(93.75%)	12/16(75%)	13/16(81.25%)	13/16(81.25%)

TABLE - 5 COMPARISON OF COMBINED DISC TEST WITH E TEST

COMBINED DISC TEST	E TEST		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	13	0	13
NEGATIVE	2	0	2
TOTAL	15	0	15

Sensitivity - 86.67 %

PPV – 100 %

Accuracy – 86.67%

TABLE - 6 COMPARISON OF DOUBLE DISC SYNERGY TEST WITH E TEST

DOUBLE DISC SYNERGY TEST	E TEST		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	13	0	13
NEGATIVE	2	0	2
TOTAL	15	0	15

Sensitivity - 86.67 %

PPV – 100 %

Accuracy – 86.67 %

TABLE - 7 COMPARISON OF MODIFIED HODGE TEST WITH E TEST

MODIFIED HODGE TEST	E TEST		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	12	0	12
NEGATIVE	3	0	3
TOTAL	15	0	15

Sensitivity - 80%

PPV – 100 %

Accuracy – 80 %

TABLE - 8 COMPARISON OF E TEST AND MBL PRODUCTION

E TEST	MBL PRODUCERS	NON PRODUCERS	TOTAL
POSITIVE	15	0	15
NEGATIVE	0	1	1
TOTAL	15	1	16

Sensitivity - 100%

Specificity - 100 %

PPV – 100 %

NPV – 100 %

Accuracy – 100%

TABLE - 9 PREVALENCE OF MBL

TOTAL ISOLATES	MBL DETECTED	PREVALENCE OF MBL
211	15	7.11%

TABLE - 10 PREVALENCE OF MBL AMONG MEROPENEM RESISTANT ISOLATES

ORGANISM	NO OF MRP RES ISOLATES	MBL PRODUCTION	
		NO	%
P. aeruginosa	16	15	93.75

TABLE - 11 AGE WISE DISTRIBUTION OF CASES

AGE (YEARS)	MBL	NON MBL	TOTAL
0-10	0	30 (15.31%)	30 (14.22%)
11-20	2(13.33%)	12 (6.12%)	14 (6.64%)
21-30	3(20%)	23 (11.73%)	26 (12.32%)
31-40	3(20%)	25(12.76%)	28 (13.27%)
41-50	3(20%)	27(13.78%)	30 (14.22%)
51-60	0	33(16.84%)	33 (15.64%)
>60	4(26.67%)	46(23.46%)	50 (23.69%)
TOTAL	15(100%)	196(100%)	211(100%)

TABLE – 12 SEX WISE DISTRIBUTION OF CASES

SEX	MBL	NON MBL	TOTAL
MALE	9(60%)	134(68.37%)	143(67.77%)
FEMALE	6(40%)	62(31.63%)	68(32.23%)
TOTAL	15(100%)	196(100%)	211(100%)

SEX RATIO OF MALE : FEMALE AMONG P. aeruginosa is 2.1 : 1

SEX RATIO OF MALE : FEMALE AMONG MBL isolates is 3 : 2

TABLE – 13 AGE & SEX WISE DISTRIBUTION OF CASES

AGE IN YEARS	SEX		TOTAL
	MALE No & %	FEMALE No & %	
0-10	18(12.59%)	12(17.65%)	30 (14.22%)
11-20	9(6.29%)	5(7.35%)	14 (6.64%)
21-30	18(12.59%)	8(11.77%)	26 (12.32%)
31-40	20(13.99%)	8(11.77%)	28 (13.27%)
41-50	21(14.69%)	9(13.24%)	30 (14.22%)
51-60	28(19.58%)	5(7.34%)	33 (15.64%)
61-70	10(6.99%)	14(20.59%)	24 (11.37%)
71-80	16(11.19%)	7(10.29%)	23(10.9%)
>81	3(2.09%)	0	3 (1.42%)
TOTAL	143(100%)	68(100%)	211(100%)

TABLE - 14 INPATIENT OUTPATIENT DISTRIBUTION OF CASES

TYPE	MBL	NON MBL	TOTAL
INPATIENT	14(93.33%)	185(94.39%)	199 (94.31%)
OUTPATIENT	1(6.67%)	11(5.61%)	12 (5.69%)
TOTAL	15(100%)	196(100%)	211(100%)

TABLE – 15 WARD WISE DISTRIBUTION OF CASES IN OUR HOSPITAL

WARD	MBL	NON MBL	TOTAL
SURGERY	6(40%)	60(30.61%)	66(31.28%)
ORTHOPAEDIC	4(26.67%)	44(22.45%)	48(22.75%)
MEDICINE	1(6.67%)	34(17.35%)	35(16.59%)
ICU(MED + PAED)	0	24(12.24%)	24(11.37%)
OPD	0	11(5.61%)	11(5.21%)
OG	1(6.67%)	8(4.08%)	9(4.27%)
UROLOGY	1(6.67%)	5(2.55%)	6(2.84%)
ENT	0	5(2.55%)	5(2.37%)
BURNS	2(13.33%)	2(1.02%)	4(1.9%)
SKIN	0	3(1.53%)	3(1.42%)
TOTAL	15(100%)	196(100%)	211(100%)

TABLE – 16 SAMPLE WISE DISTRIBUTION OF CASES

SAMPLE	MBL	NON MBL	TOTAL
PUS	11(73.33%)	120(61.22%)	131(62.09%)
SPUTUM	0	30(15.31%)	30(14.22%)
URINE	1(6.67%)	20(10.21%)	21(9.95%)
BLOOD	0	18(9.18%)	18(8.54%)
AURAL SWAB	0	5(2.55%)	5(2.37%)
BURNS WOUND	2(13.33%)	2(1.02%)	4(1.9%)
CATHETER TIP	1(6.67%)	0	1(0.47%)
ET TUBE	0	1(0.51%)	1(0.47%)
TOTAL	15(100%)	196(100%)	211(100%)

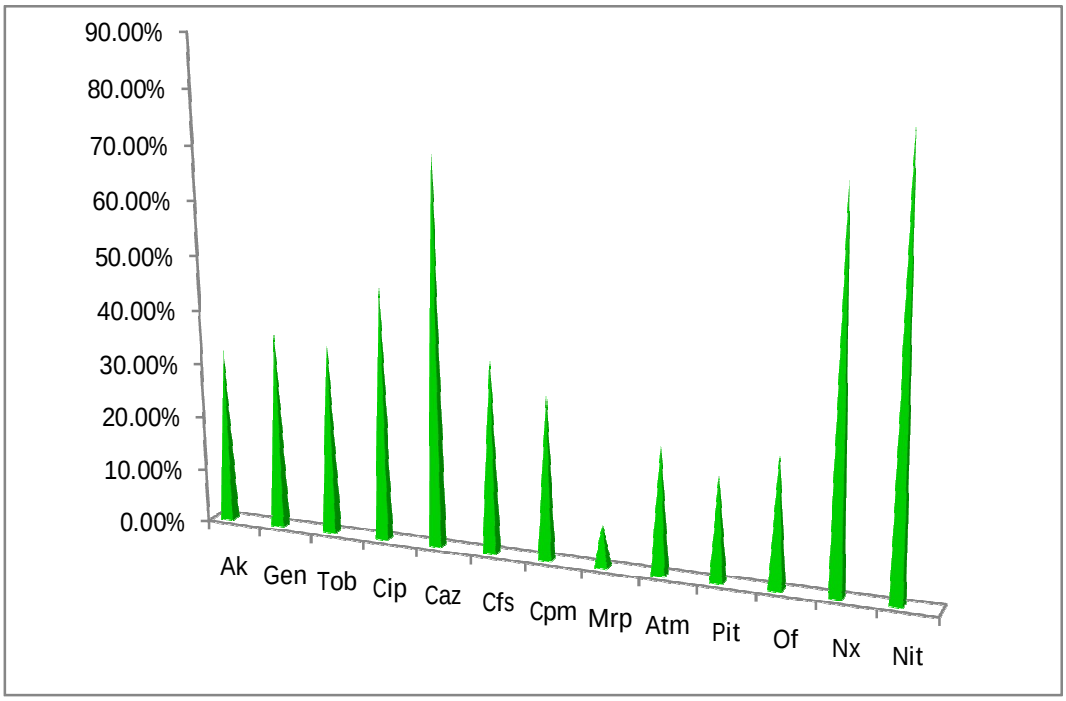
TABLE – 17 INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA ISOLATES

INFECTIONS	MBL	NON MBL	TOTAL
CELLULITIS	3(20%)	39(19.9%)	42(19.9%)
TRAUMA WOUND INFECTION	4(26.67%)	35(17.86%)	39(18.47%)
DIABETIC FOOT ULCER	3(20%)	32(16.33%)	35(16.59%)
PNEUMONIA	0	30(15.31%)	30(14.22%)
URINARY TRACT INFECTION	1(6.67%)	17(8.67%)	18(8.54%)
FEVER	0	12(6.12%)	12(5.69%)
SEPTICEMIA	1(6.67%)	10(5.1%)	11(5.21%)
NECROTISING FASCITIS	0	9(4.59%)	9(4.27%)
POST OP	1(6.67%)	5(2.55%)	6(2.84%)
CSOM	0	5(2.55%)	5(2.37%)
BURNS	2(13.32%)	2(1.02%)	4(1.9%)
TOTAL	15 (100%)	196 (100%)	211(100%)

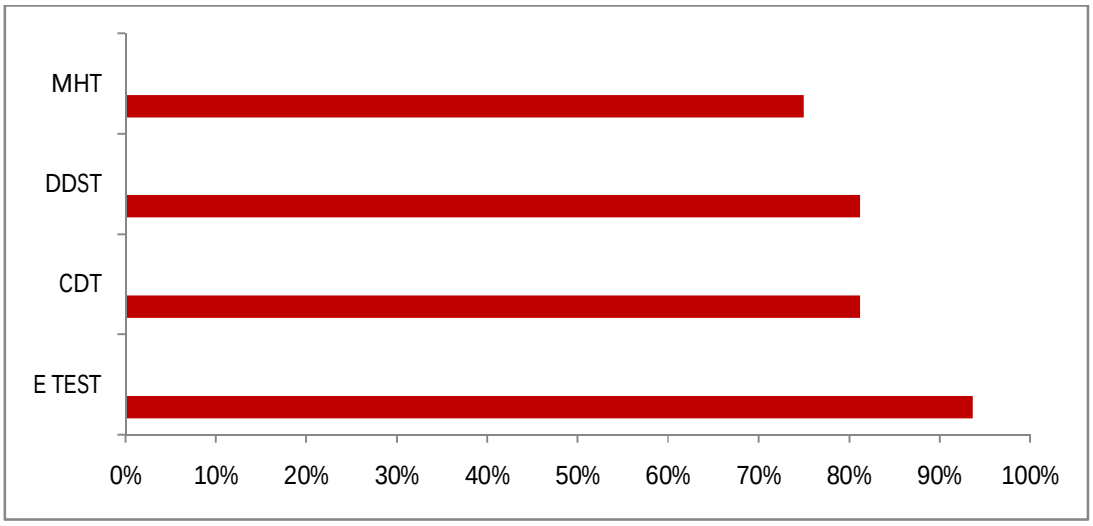
TABLE - 18 : ANTIBIOTIC RESISTANCE PATTERN OF METALLOBETALACTAMASE PRODUCING ISOLATES:

ANTIBIOTICS	MBL PRODUCERS (n = 15)	
	NO	PERCENTAGE
AMIKACIN	10	66.67%
GENTAMICIN	1	73.33%
TOBRAMYCIN	15	100%
CIPROFLOXACIN	1	73.33%
CEFTAZIDIME	15	100%
CEFAPERAZONE SULBACTUM	15	100%
CEFIPIME	15	100%
MEROPENEM	15	100%
AZTREONAM	13	86.67%
PIPERACILLIN TAZOBACTUM	9	60%
AMONG URINE ISOLATES:		
OFLOXACIN	1	100%
NORFLOXACIN	1	100%
NITROFURANTOIN	1	100%

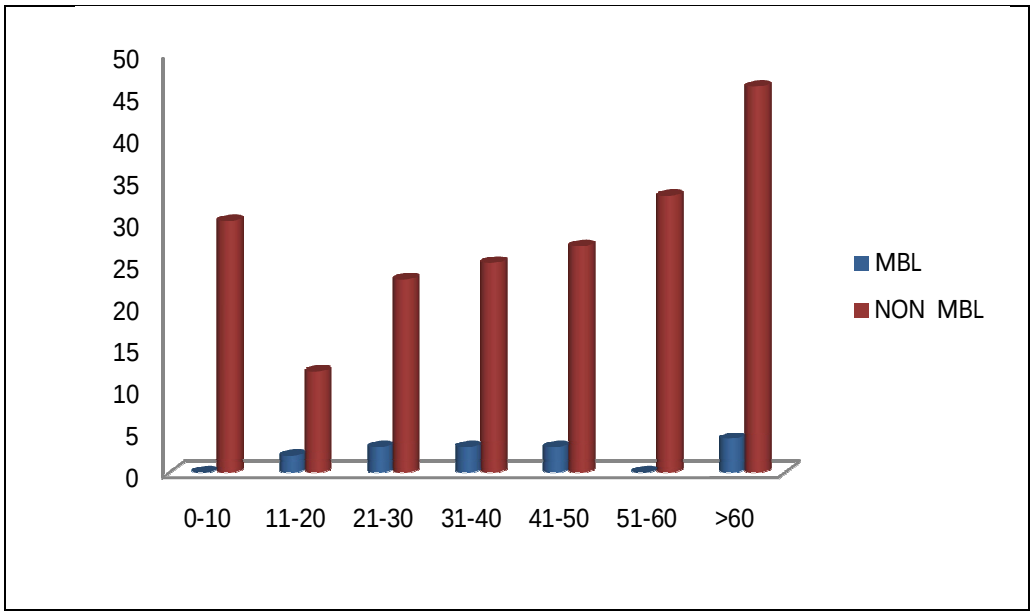
1) RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES



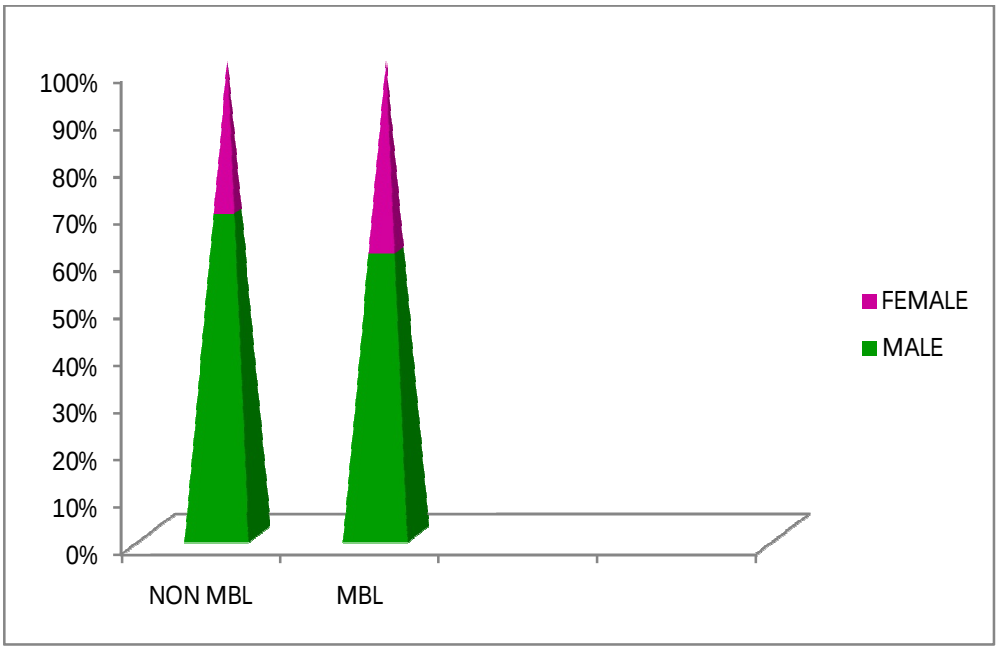
2) MBL DETECTION BY DIFFERENT PHENOTYPIC METHODS



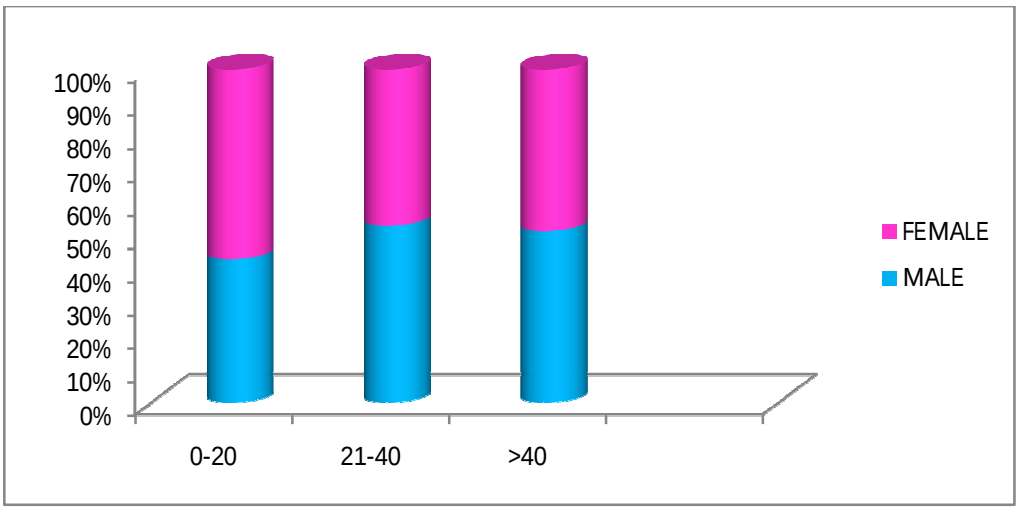
3) AGE WISE DISTRIBUTION OF ISOLATES



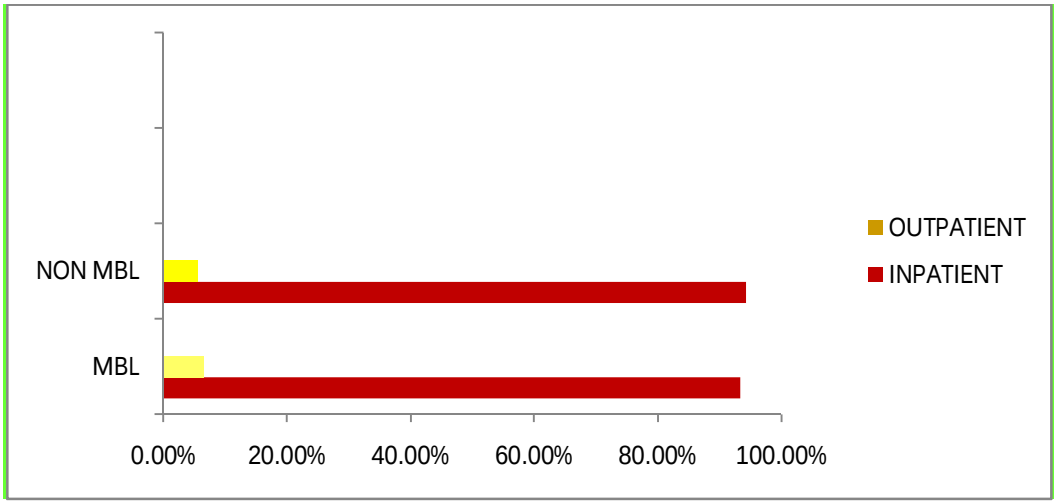
4) SEX WISE DISTRIBUTION OF ISOLATES



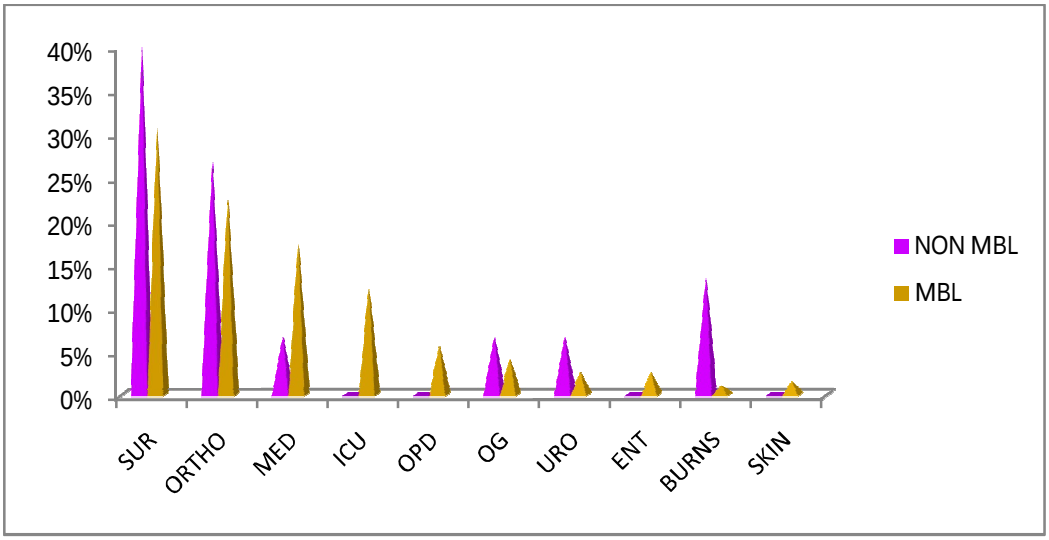
5) AGE AND SEX WISE DISTRIBUTION OF PSEUDOMONAS AERUGINOSA ISOLATES



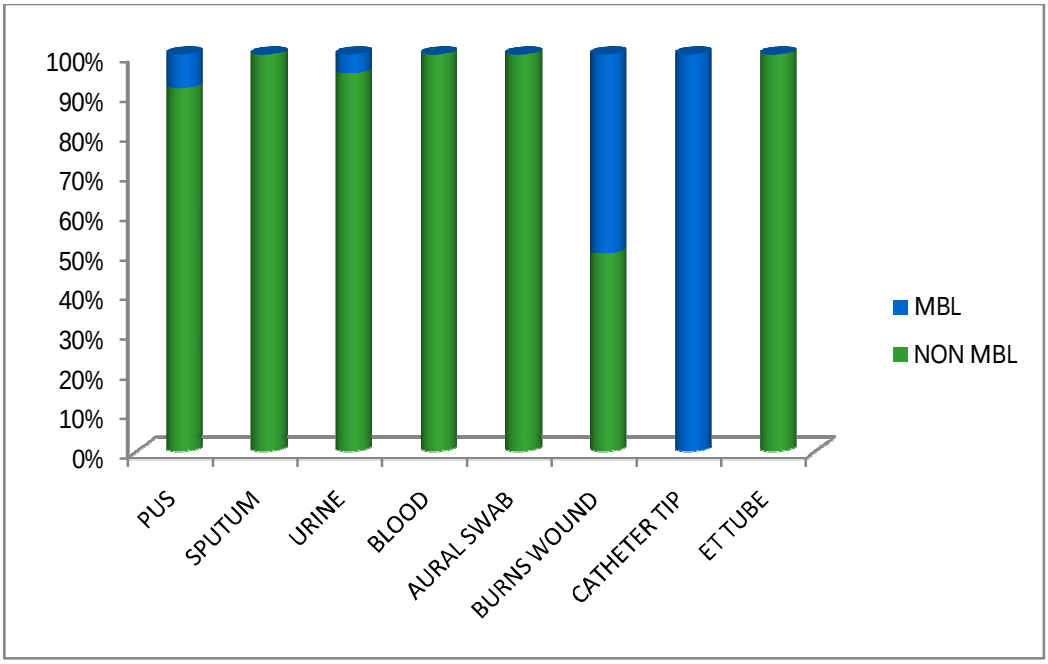
6) IP & OP DISTRIBUTION OF PSEUDOMONAS AERUGINOSA ISOLATES



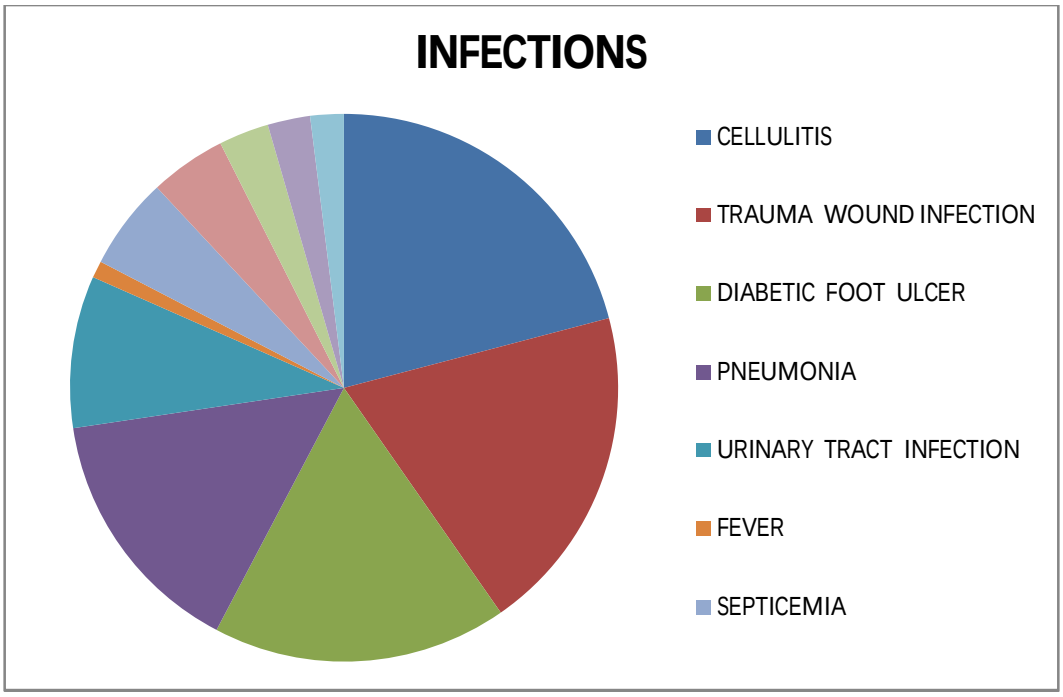
7) WARD WISE DISTRIBUTION OF ISOLATES



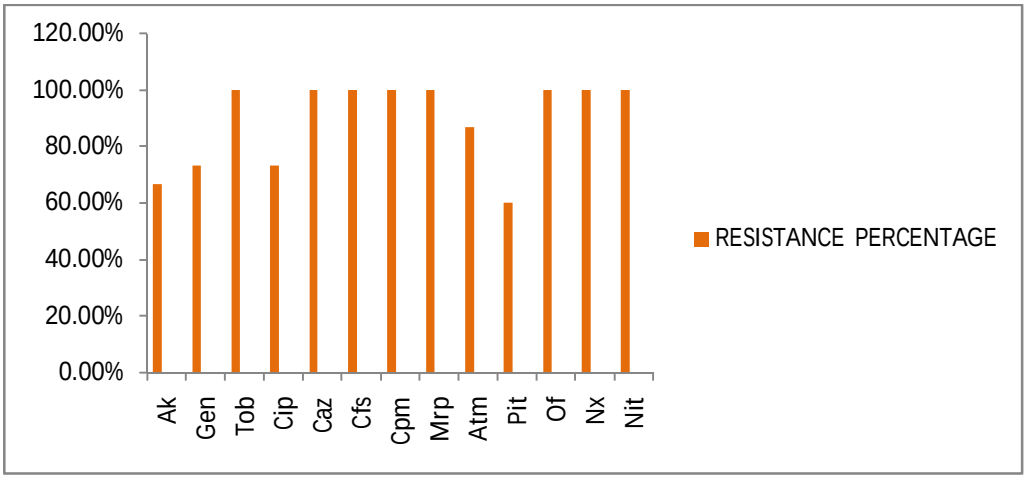
8) SAMPLE WISE DISTRIBUTION OF ISOLATES



9) INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA ISOLATES



10) ANTIBIOTIC RESISTANCE PATTERN IN MBL ISOLATES



DISCUSSION

DISCUSSION

There is higher morbidity and mortality associated with MBL producing organisms because of the fact that all classes of betalactam antibiotics will be hydrolysed by MBL. As we have not yet developed a safe therapeutic antibiotic, there will be a clinical disaster if it spreads continuosly. The presence of an MBL positive isolate causes a therapeutic problem and is also of major concern in the infection control management. Because MBL producing strains are difficult to detect, they pose a serious threat as it can spread silently within institutions. It also has the important role in horizontal MBL gene transfer to other pathogens in the hospital. The call of the hour now is detection and containment and not mere treatment.

Hence with the above perspective, the present study was conducted an attempt to know the prevalence of MBL producing P. aeruginosa isolated from sputum, wound swabs, blood, catheter tip, urine and aural swabs and also their antibiogram at Coimbatore Medical College, Coimbatore. The results are compared with other studies.

Age wise Distribution:

In this study the predominant age group is above 60 years both for P. aeruginosa and MBL isolates. This is similar to the study by Deeba et al[42] and Vinodkumar et al[51] in whose studies the most common age group is more than 60 years. It is different from Prabhat et al[52] and in his study among post operative wound infections the most common age group is 21 - 40 years. Among MBL isolates the isolates are mostly observed in the age group of above 40 years. This is also seen in the study by Shobha et al[53] and Varaiya et al[39].

Sex wise distribution:

In our study males (67.77%) are predominantly affected than females among P. aeruginosa. This is similar to the study by Prashant et al[54], Rajat et al[55] in whose study 64.28% and 61% were males. In the study by Shobha[53] et al males are more susceptible than females but with a higher percentage about 81%. The male female ratio in our study is 2.1 : 1. It correlates well with the study by Luqman et al[56] and Shampa et al[38]. In MBL producers the male female ratio is 3 : 2. It is similar to the study by Varaiya et al[39] and Shobha et al[53]. In our study males are commonly affected in

the age group of above 40 years. This is similar to the study by Senthamarai et al[57] in whose study males are commonly observed above 40 years age group.

Out/In Patient distribution:

This study shows that the isolates of P. aeruginosa as well as MBL isolates are more frequently distributed among the inpatients (94.31%) than the outpatients. This correlates well with the following studies by Bogiel et al[58], Vikas umar et al[59] and Patil et al[60] which showed 92.9%, 87% and 72% respectively.

Ward wise distribution:

Surgery is the most frequent ward (31.28%) from which the P. aeruginosa isolates are obtained in our study. This is similar to the study by Angadi et al[61], Rajat et al[55] and Deeba et al[42] which showed 36%, 48% and 60% respectively. Ortho is the second most common ward which correlates with the study by Prashant et al[54] . But it differs from the study by Wankhede et al[62], Nasrin et al[63] in whose study ICU is the most frequent ward among the isolates studied. Even among MBL isolates surgery is the most common ward (40%) which is similar to the study by Sherin et al[64], Attal Roa et al[36] and Deeba et al[42] which showed 51%, 50% and 36%. Ortho is

the second ward similar to the study by Mehul et al[27] among MBL isolates.

Sample Wise Distribution:

In our study wound swab (62.09%) is the most common specimen among the isolates of P. aeruginosa. This is also observed in the following studies by Tellis et al[65], Anil Rajput et al[7] , Attal Roa et al[36] and Angadi et al[61]. But Burns wound is the most frequent specimen obtained by the study by ElBarky et al[66] which showed 37.93% and Eman et al[67] which showed 67%; Urine is the predominant specimen in the study by Nutan et al[67]; Sputum is predominant isolate by Sangeetha et al[68] study. Among MBL isolates Wound swab is the predominant specimen (73.33%) obtained in our study. This correlates well with the study by Ahir et al[69], Bhaleroa et al[70]. But in the study by Bogiel et al[58] and Wankhede et al urine and in the study by Shanthi et al[35] Respiratory tract specimen are the frequently obtained specimen respectively among MBL producers.

Distribution of P. aeruginosa infections:

The predominant infections caused by P. aeruginosa in our study are cellulitis (19.9%), wound infections caused by trauma (18.47%) and diabetic foot infections (16.59%). This is

similar to the study by Prashant et al[54] which showed that among the infections, 19 % were cellulitis, 16.67 % trauma wound infections, 15 % diabetic foot ulcers. It differs from the study by Mehul et al[27] in whose study predominant infections are burn wounds, ulcers and abscesses each constituting 25 %.

Distribution of Antibiotic Sensitivity Pattern:

The following are Pseudomonas aeruginosa isolates resistance patterns: Ceftazidime is 71.09 % resistant similar to the study by Ahir et al[69], Behera et al[37] and Luqman et al[56]; Ciprofloxacin is 46.45% resistant similar to study by Eman et al[46], Prashant et al[54] and Rajat et al[55]; Gentamicin is 36.02 % and Amikacin is 32.23 % resistant which correlates with the study by Senthamarai et al[57], Prashant et al[54] and Basak et al[71]; Tobramycin is 35.07% resistant similar to study by Mohammed et al[45], Sanchez et al[72] and Prashant et al[54]; Cefaperazone sulbactum is 35.07% similar to Senthamarai et al[57] study; Cefipime is 29.86 % resistant which correlates with the study by Varaiya et al[39], Mohammed et al[45] and Prabhat et al[52]; Aztreonam is 23.225 resistant which is similar to the study by Zubair et al[73], Mohammed et al[45] and Basak et al[71]; Piperacillin Tazobactum is 18.96 % resistant similar to study by Prabhat et al[52] and Basak et

al[71]. Among the urine isolates Ofloxacin is 23.8 % resistant which correlates with Senthamarai et al[57] study; Norfloxacin is 71.43 % which is similar to the study by Bhalerao et al[70] in which it is 73% and Nitrofurantoin is 81 % resistant which is slightly lower than the study by Bhalerao et al[70] . Thus the prevalence and sensitivity pattern of P. aeruginosa can vary within the community or between hospitals located in the same community or vary between the patients in the same hospital. Hence it is the duty of the physician to know about the prevalence as well as antibiotic susceptibility pattern of the frequently encountered organisms. So there should be a system in each hospital for the surveillance of antimicrobial resistance which has to collect and collaborate the microbiological and clinical data.

Prevalence of MRP Resistance:

Meropenem resistance in our study is 7.58% which is similar to the study conducted by Moyo et al[74], Mehul et al[27] , Basak et al[71] and Attal Roa et al[36] which showed 8.88%, 5%, 2.4% and 12.9% . But it is of higher values in the following study by Kali et al[76], Buchunde et al[77] and Supriya et al[78] which may be due to the usage of broad spectrum antibiotics in large number to the patients. In our

study the percentage is less which signifies that the broad spectrum antibiotics were used conservatively.

Prevalence of MBL:

MBL prevalence among P. aeruginosa isolates is 7.11%. This is similar to the study conducted by Navaneeth et al[79], Agarwal et al[80], Buchunde et al[77]. This prevalence is increased in the study by Vahdani et al[81], Oh EJ et al[82]. The high metallobetalactamase prevalence in the above said studies is due to the indiscriminate use of carbapenems in that part of the world. This prevalence is decreased in the study by Wolska et al[83].

Resistance of MBL among MRP Resistant isolates:

In our study MBL resistance among MRP resistance isolates is 95% which is similar to the study conducted by Vinod kumar et al[51], Sakshi et al[84], Attal Roa et al[36] and Vinita et al[85]. This percentage is decreased in the study conducted by Perez et al[86], Samuelsen et al[87] and Franco et al[88].

MIC of MRP:

The Minimum Inhibitory Concentration of Meropenem resistant isolates are in the range from 24 – 256 µg / ml. This correlates well with the study by Sherin et al[64], Jayakumar et al[89] and Deeba et al[42].

Antibiotic Resistance pattern among MBL isolates:

Among the metallobetalactamase producing Pseudomonas aeruginosa isolates Ceftazidime, Cefaperazone sulbactum, Cefipime, Tobramycin and Meropenem showed 100 % resistance. This correlates well with the study by Varaiya et al[39], Deeba et al[42], Shoba et al[53] and Attal Roa et al[36]. Gentamicin and Ciprofloxacin showed 73.33 % resistance which is similar to the study by ElBarky et al[66]. Aztreonam showed 86.67 % resistance which is in accordance with the study by ElBarky et al[66]. Amikacin showed 66.67 % resistance which correlates with the study by Sherin et al[64]. Piperacillin Tazobactum showed 60 % resistance which is similar to the study by Sherin et al[64] and Varaiya et al[39]. Among urine isolates Ofloxacin, Norfloxacin and Nitrofurantoin showed 100 % resistance. This is in accordance with the study by Bhalero et al[70], Mouawad et al[90] and Deeba et al[42] which showed 100 % resistance to Nitrofurantoin and Ofloxacin.

Detection of MBL by Phenotypic Tests:

Among the Meropenem resistant isolates, 93.75 % of MBL were detected by E test method. This is in accordance with the study by Basak et al[71], Varsha et al[91] and Aktas et al[92]. By using Modified Hodge test method 75 % of MBL were detected. This is similar to

the study by Sakshi et al[84] and Attal Roa et al[36]. With Double Disc synergy test method 81.25 % were MBL producers. It correlates with the study by Sherin et al[64] and Basak et al[71]. By using combined Disc test method 81.25 % were MBL producers. This corresponds with the following study by Clare et al[93], Sangeetha et al[68], Behera et al[37], Vikas kumar et al[59] and Hemalatha et al[75].

Sensitivity, Specificity, PPV, NPV and Accuracy of four tests:

In our study Modified Hodge test shows 80 % sensitivity, 100 % Positive predictive value and 80 % accuracy. But in the study by Tellis et al[65] it showed a sensitivity of 74.32 % and 98.2 % of PPV. By Combined disc test, the sensitivity, PPV and Accuracy were 86.67 %, 100 % and 86.67 % respectively. This corresponds to the study by Manoharan et al[41] and Maria et al[94]. But in the study by van der Bij et al[95] there was 100 % sensitivity to combined disc test and Samuelson et al[87] showed 29 % of PPV. Double disc synergy test shows sensitivity of 86.67 %, PPV of 100 % and Accuracy of 86.67% . This is similar to the study by Tellis et al[65] and Clare et al[93]. It is different from the study by Khosravi et al[96] which shows 100 % sensitivity.

In our study E test showed accurate results with excellent sensitivity and specificity. This correlates with the study by Timothy et al[97] and Davies et al[98]. Both DDST and CDT are the better tests than Modified Hodge test in our study. This is similar to the studies by Jesudason et al[99], Yan et al[100] and Lee et al[50] which states that Double Disc Synergy Test is better than Modified Hodge test. In the study by Sevitha Bhat et al[101], DDST is a better test. So E test is a simple test to perform as well as to interpret. This test can be proposed to the clinical laboratories to screen for metallobetalactamases in Meropenem resistant P. aeruginosa. As E test has high specificity it can be used to detect MBLs in low prevalence areas also.

SUMMARY

SUMMARY

The present study was carried out at Coimbatore Medical College and Hospital, Coimbatore from August 2013 - July 2014. Among the clinical isolates of Pseudomonas aeruginosa obtained from various samples, antibiotic resistance was found and Metallobetalactamase production was detected by four phenotypic methods.

- The maximum number of Pseudomonas aeruginosa isolates are in the age group of above 60 years.
- This study showed male preponderance with the male female ratio of 2.1 : 1.
- In our study the isolates were mostly obtained from the inpatients.
- Wound swab is the predominant specimen in the isolates, in this study.
- This study showed surgery is the most common ward from where isolates are obtained.
- Cellulitis is the most observed infection among P. aeruginosa isolates.

- Overall Ceftazidime (71.09%) is the most resistant drug followed by Ciprofloxacin (46.45%) and Gentamicin (36.02%). Among urine isolates Nitrofurantoin shows 81% resistance.
- The MRP Resistance among our isolates is 7.48%
- The Prevalence of Metallobetalactamase among P. aeruginosa isolates is 7.11%.
- Among MRP Resistance isolates 95% were MBL producers.
- The MIC of MRP resistant isolates is in the range of 24 - 256 µg/ml.
- Among the four phenotypic tests the maximum sensitivity of 100 % and the maximum specificity of 100 % was obtained for E test, the maximum positive predictive value of 100 % was obtained for DDST, CDT, MHT, E TEST i.e. all the four phenotypic tests. The maximum negative predictive value of 100 % was obtained for E test and again the maximum accuracy of 100% was obtained for E test only.
- Among the phenotypic tests E Test is the test by which maximum number of MBL producers were detected.

- The MBL isolates are predominantly seen in the age group of above 40 years.
- Among MBL isolates males are more frequently affected. Male female ratio is 3 : 2 .
- From surgery ward MBL producers are commonly isolated.
- Among MBL producers 73.33 % were pus swabs, 13.33% were from burn wounds.
- Cellulitis is often encountered in MBL producers.
- The antibiotics which are 100 % resistant in MBL producers are Tobramycin, Ceftazidime, Cefaperazone sulbactum, Cefepime and Meropenem. Among urine isolates 100 % resistance was obtained for Ofloxacin, Norfloxacin and Nitrofurantoin.

CONCLUSION

CONCLUSION

Among hospital isolates of P. aeruginosa, Metallo betalactamase producing organisms are becoming a significant problem. There is increased prevalence of ESBL producing organisms in the hospitals nowdays and the carbapenem usage is also increased which leads to the problem of increased MBL production. In this era, the emergence of Metallobetalactamase has led to the requirement of strict statuatory guidelines to intervene and limit the inappropriate use of antibiotics.

The following are the possible factors which leads to unnecessary antimicrobial usage. i) No guidelines to implement the rational antibiotic prescribing principle. ii) Ignorance of the rise in multidrug resistant organisms which is an alarming problem now. iii) Promotion of pharmaceutical products. iv) Infection control is inadequate and it further compounds the problem.

As we have to monitor these emerging resistant determinants in large scale, we are in the need to develop a simple

screening test designed to detect MBL production. There are various screening methods recommended to detect MBL production.

In our study 95 % of MRP resistant isolates are MBL producers by E test method. Still some other resistance mechanisms like permeability mutation or porin loss or upregulation of efflux systems can be missed by the E test. If we detect MBL producing P. aeruginosa isolates earlier, appropriate antimicrobial therapy can be provided to the patient and thereby we can avoid the development as well as dissemination of these multidrug resistant strains. There is very few development of newer antibiotics and it also takes more time to be available commercially. The only alternative left is stringent infection control plus antibiotic stewardship program which limits the spread of metallobetalactamase producing organisms by using the available antimicrobial armamentarium.

In our country the hospitals are woefully inadequate in proper implementation of hospital control policies. Proper disinfection practices can reduce infection. Antibiotics are prescribed as a prophylactic measure during the postoperative period. Even after the antibiotics are administered for the required period, many times the stop signals are not given.

Detection of genes which code for metallobetalactamase production by PCR gives satisfactory and reliable results. But because of the cost this method is of limited practical value in clinical laboratory for daily use.

So E test, a simple and inexpensive method must be introduced in every clinical microbiological laboratory in order to aid in infection control. In case of very small laboratories in whom E Test itself is costlier, they can use Combined disc test or Double disc synergy test to detect MBL production.

BIBLIOGRAPHY

BIBLIOGRAPHY

1) Strateva T , Yordanov D. Pseudomonas aeruginosa - a phenomenon of bacterial resistance J Med Microbiol. 2009 Sep;58(Pt 9):1133-48.

2) Robert E.W. Hancock,1 David P. Speert2 Regulation of Chromosomally Encoded Resistance Mechanisms Clin Microbiol Rev. Oct 2009; 22(4): 582–610.

3) Johann D.D. Pitout, Daniel B.Gregson, Laurent Poirel, Jo-Ann McClure, Philip Le and Deinrdre L.Church,Detection Of Pseudomonas aeruginosa Producing MBL in a Large Centralised Laboratory, J Clin Microbiol. 2005 July; 43(7):3129-3135.

4) K Lolans, A.M.Queenan, K.Bush, A.Sahud and J.P.Quinn, First nosocomial Outbreak of Pseudomonas aeruginosa Producing an Integron-Borne metallobetalactamase(VIM-2) in the United States, Antimicrobial Agents Chemotherapy,2005,August; 49(8):3538-3540.

5) David M,Livermore, Multiple Mechanisms of Antimicrobial Resistance in Pseudomonas aeruginosa: our worst Nightmare?, Clin Infect Dis,(2002):34(5),P 634-640.

6) Connie R.Mahon, Donald C.Lehman, George Manuselis – Textbook of Diagnostic Microbiology,Third Edition , Page -571,572.

7) Dr.Anil Rajput, Dr.Bhavin Prajapati, Dr.Bhimal Chauhan, Dr.Atit Shah, Dr.Toral Trivedi, Dr.Mina Kadam, Prevalence of Metallobatalactamases (MBL) producing Pseudomonas aeruginosa in a Tertiary care Hospital,Indian Journal of basic & Applied Medical Research:September 2012:Vol-1, Issue-4, P304-308.

8) Yoshichika Arakawa, Naohiro Shibata, Keigo Shibayama, Hiroshi Kurokawa, Tetsuya Yagi, Hiroshi Fujiwara and Masafumi Goto, Convenient Test for Screening Metallo- β- Lactamase Producing Gram-Negative Bacteria by Using Thiol Compounds, J Clin Microbiol. 2000 January; 38(1):40-43.

9) Mandell,Douglas and Bennett's Principles and Practice of Infectious Diseases,7th edition,Volume 2, Page 2835.

10) Ananthanarayanan & Panicker's Textbook Of Microbiology,9th Edition, Page 314 – 316.

11) Greenwood, Barer, Slack, Irving-Medical Microbiology,18 th edition,Page 298.

12) Topley & Wilson's Microbiology & Microbial Infections,Bacteriology-Volume 2,Page 1591.

13) Koneman's Color Atlas and Textbook of Diagnostic microbiology,Sixth Edition.Page 319,320.

14) Murray,baron,Jorgensen.landry,Pfaller, Manual of Clinical Microbiology,vol 1,9th edition,Page 734,735.

15) Javetz, Melnick & Adelberg's Medical Microbiology,25th edition. Pages 227,228.

16) Myer's and Koshi's Manual of Diagnostic Procedures in Medical Microbiology and Immunology, CMC, Vellore.Page -206,209.

17) Prescott's Microbiology.Willey,Sherwood,Woolverton,8th edition,Page 200(?534)

18) Bailey and Scott's Diagnostic Microbiology,Thirteenth Edition.Pages 336 – 343.

19) P A Lambert, Mechanisms Of Antibiotic Resistance In Pseudomonas aeruginosa, Journal of the royal society of medicine, 2002;95(Suppl. 41):22–26.

20) Gorbach,Bartlett,Blacklow,Infectious diseases,3rd edition,Page1704-12.

21) Mahon,Lehman,Manuselis,Textbook of Diagnostic microbiology,3rd edition,Page 574.

22) Mackie & Mccartney Practical Medical Microbiology,Thirteenth Edition,Vol 2, Page 495,502

23) Satoskar, Rege, Bhandarkar, Pharmacology and Pharmacothgerapeutics,Revised 22 nd edition,page 684.

24) Goodman & Gilman"s The Pharmacological Basis of Therapeutics,12th edition,Page 1516.

25) Fauci, Braunwald, Kasper, Hauser, Longo, Jameson, Loscalzo, Harrison's principles of internal medicine, 18th edition,page 734.

26) Varsha gupta ,Shailpreet Sidhu and Jagadish Chander.Metallo-β-lactamase producing nonfermentative gramnegative bacteria:An increasing clinical threat among hospitalized patients. Asian Pac J Trop Med. 2012 Sep;5(9):718-21.

27) Mehul S chaudari,Tanuja b Javadekar,govind Ninama,Neelam Pandya and Jivraj Damor.A study of MBL producing Pseudomonas aeruginosa in clinical samples of SSG Hospital.National Journal of Medical Research.Oct-Dec 2011,1(2):60-63.

28) U chaudary and R Aggarwal.Extended spectrum beta lactamases-an emerging threat to clinical therapeutics.IJMM,2OO4;22(2):75-80.

29) Pawe Sacha, PiotrWieczorek, Tomasz Hauschild, Marcin orawski, Dorota Olszanska, Elbieta Tryniszewska, Metallo-β-lactamases of Pseudomonas aeruginosa - a novel mechanism resistance to β-lactam antibiotics, Folia Histochem Cytobiol. 2008:46(2): 137 (137-142).

30) Misra RN. Metallo β-lactamases: A perspective and implications. Med J DY Patil Univ 2012;5:10-3.

31) Kyungwon Lee, Jong Back Lim, Jong Hwa Yum, Dongeun Yong, Yunsop Chong, June Myung Kim, and David M. Livermore bla $_{VIM\ 2}$ Cassette-Containing Novel Integrons in Metallo-β-Lactamase-Producing Pseudomonas aeruginosa and Pseudomonas putida Isolates Disseminated in a Korean Hospital, Antimicrob Agents Chemother. Apr 2002; 46(4): 1053–1058.

32) Gary D. Overturf, MD, Carbapenemases A Brief Review for Pediatric Infectious Disease Specialists, The Pediatric Infectious Disease Journal, Volume 29, Number 1, January 2010,Page 68-70.

33) Patel G and Bonomo RA (2013) “Stormy waters ahead”: global emergence of carbapenemases. Front. Microbiol. 4:48.

34) Timothy R Walsh,Mark A Toleman,Laurent poirel and Patrice Nordmann.metallo-β-lactamases:the quiet before the storm?Clin.Microbiol.Rev.Apr 2005;18(2):306-325.

35) M Shanthi and Uma Shekar.Multi drug resistant Pseudomonas aeruginosa and Acinetobacter baumanii infections among hospitalized patients:risk factors and outcomes.Journal of Assoiation of physicians of India.2009;57:636-645.

36) Attal RO,Basak S and Mallick SK and Bose S.Metallobetalactamase producing Pseudomonas aeruginosa:An emerging threat to clinicians.Journal of Clinical and Diagnostic Research.August 2010;4:2691-2696.

37) Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallobetalactamase producing Pseudomonas aeruginosa. Indian J Med Microbiol 2008;26:233-7.

38) Shampa Anupurba,Amitha bhattacharjee,Atul garg and Malay Ranjan Sen.Antimicrobial susceptibility of Pseudomonas aeruginosa isolated from wound infections.Indian J deramatol 2006;51(4):286-288.

39) A Varaiya,M Kulkarni,P Bhalekar and J Dogra.Incidene of Carbapenem resistant Pseudomonas aeruginosa in Diabetes and cancer patients.IJMM,2008;26(3):238-240

40) M.J.C Noyal,G.A.Menezes,B.N.Harish,S.Sujatha and S.C.Parija.Simple screening tests for detection of carbapenems in clinical isolates of gram-negative bacteria.Indian J Med Res ,June 2009;129:707-712.

41) Manoharan A, Chatterjee S, Mathai D, Benjamin M, Aggarwal A, Deotale V, et al. Detection and characterization of
metallo beta lactamases producing Pseudomonas aeruginosa. Indian J Med Microbiol 2010;28:241-4.

42) Deeba Bashir, Manzoor Ahmad Thokar, Bashir Ahmad Fomda, Gulnaz Bashir, Danish Zahoor, Shabir Ahmad and Abubaker S. Toboli, Detection of metallo-beta-lactamase (MBL)producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir.African Journal of Microbiology research.January 2011;5(2):164-172.

43) S John and Balagurunatham R.Metallo beta lactamase produing Pseudomonas aeruginosa and Acinetobacter baumanii.IJMM,2011;29(3):302-304.

44) Ibukun Aibinu,Tochukwu Nwanneka and Tolu Odughemi.Occurence of ESBL and MBL in clinical isolates of pseudomonas aeruginosa from Lagos,Nigeria.Journal of Science2007;3(4):81-85.

45) Mohamed S Ellabib, B Ph, PhD; Mohamed A Aboshkiwa, B Ph, PhD[1], Nagwa A Almargani, MSc[2] ; Zorgani AA, PhD and El-Gumati M, MD,MSc[1]
Detection and Prevalence of metallo-β -lactamase in Pseudomonas aeruginosa strains Tripoli-Libya

46) Eman M. Jarallah Mustafa J. Alammary Nosocomial outbreak of carbapenem-resistant Pseudomonas aeruginosa producing metallo beta lactamase in Al-Hilla teaching hospital, Babylon Journal of Babylon University/Pure and Applied Sciences/ No.(1)/ Vol.(22): 2012 College of Science/Babylon University Scientific Conference

47) Jean F. Mac Faddin, Biochemical Tests for Identification of Medical Bacteria, Page 51 - 54.

48) CLSI document, Clinical and Laboratory Standard Methods. Performance Standards for antimicrobial susceptibility testing: Twenty third informational supplement. Wayne PA: Clinical and Laboratory Standard Institute; 2013

49) Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem EDTA disc method for differentiation of metallo beta lactamase producing clinical isolates of Pseudomonas sp and Acinetobacter spp. J Clin Microbiol 2002;40:3798-801.

50) Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and disc synergy tests to screen metallo beta lactamase producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect 2001;7:88-91.

51) Chickmagalure Shivaswamy Vinodkumar, Srinivasa Hiresave, Basavarajappa Kandagal Giriyapal, And Nitin Bandekar, Metallo Beta Lactamase Producing Pseudomonas Aeruginosa And Its Association With Diabetic Foot, Indian J Surg. Aug 2011; 73(4): 291–294.

52) K Prabhat Ranjan, Neelima Ranjan, Satish K Bansal, And D R Arora, Prevalence Of Pseudomonas Aeruginosa In Post-Operative Wound Infection In A Referral Hospital In Haryana, India, J Lab Physicians. 2010 Jul-Dec; 2(2): 74–77.

53) Shobha Kl, Lenka Pr, Sharma M K, Ramachandra L , Bairy I .Metallo-B-Lactamase Production Among Pseudomonas Species And Acinetobacter Species In Costal Karnataka.Journal Of Clinical And Diagnostic Research 2009 October ; 3:1747-1753.

54) Prashant Durwas Peshattiwar And Basavaraj Virupaksappa Peerapur, ESBL And MBL Mediated Resistance In Pseudomonas Aeruginosa , Journal Of Clinical And Diagnostic Research. 2011 December, Vol-5(8): 1552-1554

55) Rajat Rakesh M, Ninama Govind L, Mistry Kalpesh, Parmar Rosy, Patel Kanu, Vegad Mm, Antibiotic Resistance Pattern In Pseudomonas Aeruginosa Species Isolated At A Tertiary Care Hospital, Ahmadabad, National Journal Of Medical Research,Volume 2 Issue 2 Apr – June, 2012, Page 156-159

56) Luqman Satti, Shahid Abbasi, Tanveer Ahmed Qumar, Muhammad Shoaib Khan and Zahid Ahmed Hashmi, In Vitro Efficacy of Cefepime Against Multi-Drug Resistant Pseudomonas aeruginosa – An Alarming Situation in our Setup, The Open Drug Resistance Journal, 2011, 1, 12-16.

57) Senthamarai S. ,Sunilkumar Reddy S,Sivasankari S,Anitha C,Somasunder V, Kumudavathi MS,Amsavadanisk,Venugopal V , Resistance Pattern Of Pseudomonas Aeruginosa In A Tertiary Care Hospital Of Kanchipuram, Journal Of Clinical And Diagnostic Research. 2014 May, Vol-8(5): DC30-DC32

58) Tomasz Bogiel, Aleksander Deptula,Eugenia Gospodarek,Different Methods For Detection Of Metallobetalactamases In Pseudomonas Aeruginosa Isolates,Polish Journal Of Microbiology,2010,Vol 59,No 1,45-48.

59) Vikas Kumar, Malay Ranjan Sen, Shampa Anupurba, Pradyot Prakash, Richa Gupta, An Observational Study Of Metallo Beta Lactamase Production In Clinical Isolates Of Pseudomonas Aeruginosa: An Experience At A Tertiary Care Hospital In North India, Indian J. Prev. Soc. Med. Vol. 42 No.2, 2011,Page 173-176.

60) Patil N.J,Ghosh.S.J,Shadija P.G., A Study Of Metallo-Beta Lactamase Producing Pseudomonas Aeruginosa In Patients Attending D.Y. Patil Medical College, Hospital & Research Institute, Kolhapur. Hospital Infection Control, 6-10-2013

61) Angadi KM, Kadam M, Modak MS, Batavdekar SM, Dalal BA, Jadhavvar SR, Tolpadi AD, and Shah SR."Detection of antibiotic resistance in pseudomonas aeruginosa isolates with special reference to metallobeta lactemas in a tertiary care hospital from western India" . International journal of microbiology research 2012 vol4, pp 295-298.

62) Dr. Wankhede S.V., Dr. Iyer V.S., Dr. Bharadwaj R.S., The Study Of MBL Producers In Gram Negative Isolates From ICUs And Wards, Indian Journal of Basic & Applied Medical Research; December 2011: Issue-1, Vol.-1, P. 38-46.

63) Tanzinah Nasrin MD, Shariful Alam Jilani, Lovely Barai, J. Ashraful Haq, Metallo-ß-Lactamase Producing Pseudomonas species in a Tertiary Care Hospital of Dhaka City, Bangladesh J Med Microbiol 2010; 04 (01): 43-45

64) Sherin A.S. ElMasry, Rania A. Ammar and Sally M. Saber, Phenotypic and Molecular Characterization of Imipenem Resistant pseudomonas Isolates, Life Science Journal 2012;9(2s),pages 377-383.

65) Rouchelle Tellis, Muralidharan S, Anand Ignatius Peter, Evaluation of three phenotypic methods for the detection of metallo-betalactamase production in non fermenting gram negative bacilli, International Journal of Biomedical And Advance Research, (2013) 04 (05),page 299-305.

66) Abdul ElBarky et al ,Prevalence of Extended-Spectrum Beta-Lactamase, AmpC Beta- Lactamase, and Metallo-Beta-Lactamase among Clinical Isolates of Pseudomonas aeruginosa, Journal of Advanced Biotechnology and Bioengineering, 2013, Vol 1,No. 1,pages 24-29.

67) Nutan Narayan Bhongle, Neena Vinay Nagdeo, Vilas R. Thombare. The Prevalence Of Metallo B-Lactamases In The Clinical Isolates Of Pseudomonas Aeruginosa In A Tertiary Care Hospital: An Alarming Threat. Journal of Clinical and Diagnostic Research 2012 September ,6:1200-1202.

68) Sangeetha K.T., Saroj Golia and Vasudha C.L., Phenotypic Detection Of Metallo Beta Lactamase In Gram Negative Bacterial Isolates, CIBTech Journal of Microbiology, 2014 Vol. 3 (1) January-March, pp.5-10.

69) Ahir H.R., Patel P.H., Berry R.A., Parmar R., Soni S.T., Shah P.K., Vegad M.M. And Patil S., Prevalence Of Metallo-B-Lactamases Producing Pseudomonas And Acinetobacter Species In Tertiary Care Teaching Hospital, Gujarat, International Journal of Microbiology Research, Volume 4, Issue 9, 2012, pages 322-325.

70) Bhalerao DS, Rhoushani S, Kanikeri A G, Akthar I," Study of MBL Producing Pseudomonas aeruginosa in pravara rural hospital". Pravara Med Rev 2010; 5(3).

71) Silpi Basak, Ruchita O. Attal and Monali Rajurkar (2012). Pseudomonas Aeruginosa and Newer β-Lactamases: An Emerging Resistance Threat, Infection Control - Updates, Dr. Christopher Sudhakar (Ed.), ISBN: 978-953-51-0055-3, InTech, Available from: http://www.intechopen.com/books/infection-control-updates/pseudomonasaeruginosa-and-newer-lactamases-an-emerging-resistance-threat.

72) Sanchez A,Salso S,Culebras E,Picazo J.J, Carbapnem resistance determined by metalloenzymes in clinical isolates of Pseudomonas aeruginosa,Rev ESp Quimioterap,Dec 2004,vol 17, page 336-340.

73) Zubair M , Malik A, Ahmad J.. Prevalence of metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from diabetic foot ulcer patients, Diabetes Metab Syndr. 2011 Apr-Jun;5(2):90-2.

74) S. Moyo, B. Haldorsen, S. Aboud, B. Blomberg, S. Maselle, A. Sundsfjord, N. Langeland, Samuelsen, First report of metallo-beta-lactamase producing Pseudomonas aeruginosa from Tanzania, 22nd European Congres of Clinical Microbiology and Infectious Diseases,April 2nd,2012.

75) Hemalatha V, Uma S. and Vijaylakshmi K. (2008): Detection of metallobetalactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J. Med. Researches 122:148-152.

76) Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Umadevi S, Detection of metallo-beta-lactamase producing Pseudomonas aeruginosa in intensive care units, Australas Med J. 2013 Dec 31;6(12):686-93.

77) Buchunde S, Mendiratta D K, Deotale V, Narang P. Comparison of disc and MIC reduction methods with polymerase chain reaction for the detection of metallo-β-lactamase in Pseudomonas aeruginosa. Indian J Med Microbiol 2012;30:170-4.

78) Supriya Upadhyay1, Malay Ranjan Sen1, Amitabha Bhattacharjee, Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme, J Infect Dev Ctries 2010; 4(4):239-242.

79) Navaneeth BV, Sridaran D, Sahay D, Belwadi MR (2002). A preliminary study on metallo beta-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J. Med. Res., 116: 264-267.

80) Agarwal G., Lodhi R.B., Kamalaker U.P., Khadse R.K. and Jalgaonkar S.V. (2008): Study of metallo-β-lactamase production in clinical isolates of Pseudomonas aeuroginosa. Indian J. Med. Microbial.;26(4):349-351.

81) M. Vahdani, L. Azimi, B. Asghari, F. Bazmi, and A. Rastegar Lari, Phenotypic screening of extended-spectrum ß-lactamase and metallo-ß-lactamase in multidrug-

resistant Pseudomonas aeruginosa from infected burns, Ann Burns Fire Disasters. Jun 30, 2012; 25(2): 78–81.

82) Oh EJ , Lee S, Park YJ, Park JJ, Park K, Kim SI, Kang MW, Kim BK, Prevalence of metallo-beta-lactamase among Pseudomonas aeruginosa and Acinetobacter baumannii in a Korean university hospital and comparison of screening methods for detecting metallo-beta-lactamase, J Microbiol Methods. 2003 Sep;54(3):411-8.

83) Wolska K , Jakubczak A, Soszyńska A., Antibiotic susceptibility and occurrence of ESBL, IBL and MBL in Pseudomonas aeruginosa strains, Med Dosw Mikrobiol. 2008;60(2):111-9.

84) Sakshi P. Singh, Malini Shariff Tanushree Barua & S.S. Thukral,Comparative evaluation of phenotypic tests for identification of metallo β-lactamases producing clinical isolates of Pseudomonas aeruginosa, Indian J Med Res 129, June 2009, pp 713-715.

85) Vinita Rawat, Monil Singhai, and Pankaj Kumar Verma, Detection of Different β-Lactamases and their Co-existence by Using Various Discs Combination Methods in Clinical Isolates of Enterobacteriaceae and Pseudomonas Spp, J Lab Physicians. 2013 Jan-Jun; 5(1): 21–25.

86) Perez I A , Garcia C P, Poggi M H, Braun J S, Castillo V C, Roman JC, Lagos M, Romeo O E, Porte T L, Labarca L J, Gonzalez R G., [Presence of metallo beta-lactamases in imipenem-resistant Pseudomonas aeruginosa, Rev Med Chil. 2008 Apr;136(4):423-32.

87) Samuelsen O , Buar L, Giske CG, Simonsen GS, Aasnaes B, Sundsfjord A., Evaluation of phenotypic tests for the detection of metallo-beta-lactamaseproducing Pseudomonas aeruginosa in a low prevalence country, J Antimicrob Chemother. 2008 Apr;61(4):827-30.

88) Franco R G, Caiaffa-Filho lH,Burattini M N , RossiF v(2010):Metallo-beta-lactamases among imipenem-resistant Pseudomonas aeruginosa in a brazilian university hospital, Clinics ;65(9):825- 829.

89) Jayakumar S , Appalaraju B., Prevalence of multi and pan drug resistant Pseudomonas aeruginosa with respect to ESBL and MBL in a tertiary care hospital, Indian J Pathol Microbiol. 2007 Oct;50(4):922-5.

90) R. Mouawad, C. Afif, E. Azar, E. Dahdouh, K. Masri, J. Irani and Z. Daoud, "Effect of Antibiotic Consumption on Resistance of Pseudomonas aeruginosa Isolated from Lebanese Patients with Emphasis on MBL Production," Advances in Microbiology, Vol. 3 No. 4, 2013, pp. 382-388.

91) Varsha Gupta, Shailpreet Sidhu, Jagdish Chander, Metallobetalactamase producing nonfermentative gram-negative bacteria: An increasing clinical threat among hospitalized patients, Asian Pacific Journal of Tropical Medicine (2012)718-721.

92) Aktaş Z , Kayacan CB, Investigation of metallo-beta-lactamase producing strains of Pseudomonas aeruginosa and Acinetobacter baumannii by E-test, disk synergy and PCR, Scand J Infect Dis. 2008;40(4):320-5.

93) Clare Franklin, Lisa Liolios, and Anton Y. Peleg, Phenotypic Detection of Carbapenem-Susceptible Metallo-β-Lactamase-Producing Gram-Negative Bacilli in the Clinical Laboratory, J Clin Microbiol. Sep 2006; 44(9): 3139–3144.

94) Maria Omair, Javaid Usman, Fatima Kaleem, Afreenish Hassan, Ali Khalid and Qanita Fahim, Evaluation of combined disc method for the detection of metallo-β-lactamase producing Gram negative bacilli, Malaysian Journal of Microbiology, Vol 8(1) 2012, pp. 21-25

95) van der Bij AK , Mol M, van Westreenen M, Goessens WH, Pitout JD., The laboratory diagnosis of Pseudomonas aeruginosa that produce metallo-β-lactamases in a Dutch tertiary care centre, Scand J Infect Dis. 2011 Aug;43(8):596-602.

96) Yalda Khosravi, Mun Fai Loke, Eng Guan Chua, Sun Tee Tay, and Jamuna Vadivelu, Phenotypic Detection of Metallo-β-Lactamase in Imipenem-Resistant Pseudomonas aeruginosa, Scientific World Journal. 2012; 2012: 654939

97) Timothy R. Walsh, Anne Bolmström, Anette Qwärnström, and Ana Gales, Evaluation of a New Etest for Detecting Metallo-β-Lactamases in Routine Clinical Testing, J Clin Microbiol. Aug 2002; 40(8): 2755–2759.

98) L. Davies, M. Wootton, V. E. Daniel, R. A. Howe ,Evaluation of the MBL E Test for detecting metallobetalactamase presence in Pseudomonas aeruginosa. D-739/181,50th ICAAC,Sept. 12-15, 2010 ,Boston .

99) Jesudason M.V., Kandathil A.J. and Balaji V. (2005): Comparison of two methods to detect carbapenemase &metallo-[beta]-lactamase production in clinical isolates. Indian J. Med. Res.;121: 780-783.

100) Yan JJ, Wu JJ, Tsai SH, Chaung CL (2004). Comparison of double disk, combined-disk and E-test methods for detecting metallo-betalactamases in gram negative bacilli. Diagn. Microbiol. Inf. Dis., 49: 5-11.

101) Sevitha Bhat, Renu Sharma, Zenith Euphemia, Carbapenem Resistance In Clinically Significant Non Fermenting Gram Negative Bacilli, Journal Of Evolution Of Medical And Dental Sciences,Nov 2013,Vol 2,issue 47,Page 9131-934.

ANNEXURES

(i) LIST OF TABLES

S.NO.	TABLES
1.	ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES
2.	PREVALENCE OF MEROPENEM RESISTANCE AMONG ISOLATES
3.	MIC OF MEROPENEM RESISTANT ISOLATES
4.	DETECTION OF MBL BY FOUR DIFFERENT PHENOTYPIC METHODS
5.	COMPARISON OF COMBINED DISC TEST WITH E TEST
6.	COMPARISON OF DOUBLE DISC SYNERGY TEST WITH E TEST
7.	COMPARISON OF MODIFIED HODGE TEST WITH E TEST
8.	COMPARISON OF E TEST AND MBL PRODUCTION
9.	PREVALENCE OF MBL AMONG PSEUDOMONAS AERUGINOSA ISOLATES
10.	PREVALENCE OF MBL AMONG MEROPENEM RESISTANT ISOLATES
11.	AGE WISE DISTRIBUTION OF CASES
12.	SEX WISE DISTRIBUTION OF CASES
13.	AGE & SEX WISE DISTRIBUTION OF CASES
14.	INPATIENT OUTPATIENT DISTRIBUTION OF CASES
15.	WARD WISE DISTRIBUTION OF CASES IN OUR HOSPITAL
16.	SAMPLE WISE DISTRIBUTION OF CASES
17.	INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA ISOLATES
18.	ANTIBIOTIC RESISTANCE PATTERN OF METALLOBETALACTAMASE PRODUCING ISOLATES

(ii) LIST OF CHART

S.NO	CHART
1.	RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES
2.	MBL DETECTION BY DIFFERENT PHENOTYPIC METHODS
3.	AGE WISE DISTRIBUTION OF ISOLATES
4.	SEX WISE DISTRIBUTION OF ISOLATES
5.	AGE AND SEX WISE DISTRIBUTION OF PSEUDOMONAS AERUGINOSA ISOLATES
6.	IP & OP DISTRIBUTION OF PSEUDOMONAS AERUGINOSA ISOLATES
7.	WARD WISE DISTRIBUTION OF ISOLATES
8.	SAMPLE WISE DISTRIBUTION OF ISOLATES
9.	INFECTIONS CAUSED BY PSEUDOMONAS AERUGINOSA ISOLATES
10.	ANTIBIOTIC RESISTANCE PATTERN IN MBL ISOLATES

LIST OF COLOUR PLATES

S.NO.	COLOUR PLATES
1.	Mac Conkey Agar Plate showing Pseudomonas aeruginosa
2.	Blood Agar Plate showing Pseudomonas aeruginosa
3.	Nutrient Agar Plate showing Pseudomonas aeruginosa
4.	Biochemical reactions of Pseudomonas aeruginosa
5.	Sugar fermentation Test showing Glucose Oxidation
6.	Moeller's Decarboxylase Test
7.	AST plate showing Meropenem Resistance
8.	AST plate showing Meropenem Sensitivity
9.	E Test Positive
10.	E Test Negative
11.	Combined Disc Test Positive
12.	Combined Disc Test Negative
13.	Double Disc Synergy Test Positive
14.	Double Disc Synergy Test Negative
15.	Modified Hodge Test Positive
16.	Modified Hodge Test Negative
17.	AST plate showing Ceftazidime and Cefaperazone Sulbactum Resistance

(iv) LIST OF ABBEVIATIONS

MBL	Metallo Beta lactamase
MRP	Meropenem
ESBL	Extended Spectrum Beta Lactamase
CLSI	Central Laboratory Standard Institute
PCR	Polymerase chain Reaction
LPS	Lipo Poly Saccharide
MRSA	Methicillin Resistant Staphylococcus aureus
PBP	Penicillin Binding Protein
TEM-1	Temoneira
SHV-1	Sulphydryl reagent variable
MHA	Mueller Hinton Agar
CSOM	Chronic Suppurative Otitis Media
PPV	Positive Predictive Value
NPV	Negative Predictive Value
MIC	Minimum Inhibitory Concentration
mm	millimetre
μg	microgram

v) **PROFORMA**

CASE NO:

1. Name -

2. Age -

3. Sex -

4. Date

5. OP/IP.No

6. Ward

Diagnosis:

Specimen:

Laboratory analysis:

Microscopy:

Culture:

Biochemical reactions:

Antimicrobial sensitivity:

Metallobetalactamase Detection:

1.Imipenem –EDTA double disk synergy.

2.Imipenem-EDTA combined disk test.

3.Modified Hodge test.

4. E Test.

vi) WORK SHEET

All clinical samples

↓

NLF on Mac Conkey Agar

↓

Direct Gram Stain- GNB
Oxidase test-Positive
Catalase-Positive
Motility-Motile

↓

Biochemical Reactions

↓

Pseudomonas aeruginosa

↓

Antibiogram

↓

Meropenem Resistant Isolates

↓

MBL Detection by four phenotypic methods
1] E test
2] Combined Disc Test
3] Double Disc Synergy Test
4] Modified Hodge Test

MASTER CHART

S.no	Name	Age	Sex	Sample	Ward	Diagnosis	Ak	G	Tob	Cip	Caz	Cfs	Cpm	Mrp	Azm	Pit	Of	Nor	Nit	MHT	CDT	DDST	ETEST
1	Ramalingam	35	M	Pus	SUR OPD	Necrotising Fascitis	S	R	R	R	S	R	R	S	S	S							
2	Murugesan	44	M	Pus	SUR	Diabetic Foot Ulcer	R	R	R	S	R	R	R	S	R	S							
3	Muniyammal	61	F	Sputum	MED	Pneumonia	S	S	S	R	S	S	S	S	S	S							
4	Rajeshwari	29	F	Urine	OG/Gynec OPD	UTI	S	R	S	S	R	S	R	S	S	S	R	R	R				
5	Palanisamy	75	M	Pus	ORTHO	Bed Sore	S	S	R	R	R	R	R	S	S	S							
6	Tamilarasi	45	M	Sputum	MED	Pneumonia	S	S	R	S	R	R	S	S	S	S							
7	Vijayan	52	M	Pus	SUR	DM,BK Amputation	S	R	S	R	R	S	R	S	R	S							
8	Sivamani	53	M	Pus	SUR	Post Op Wound Infection	S	S	S	R	S	S	S	S	S	S							
9	Selvam	41	M	Pus	SUR	Venous Ulcer	S	S	S	R	S	S	S	S	S	S							
10	Raman	38	M	Sputum	ORTHO	PolyTrauma with Rt Hemothorax	S	R	S	R	R	S	R	S	S	R							
11	Kamala	29	F	Pus	SUR	Post Op Wound Infection	S	R	S	R	S	S	R	S	S	S							
12	Subramani	56	M	Pus	SUR	Diabetic Foot Ulcer	S	R	R	R	R	S	R	S	R	R							
13	Murugesan	55	M	Pus	SUR	Diabetic Foot Ulcer	S	S	R	S	R	R	S	S	R	S							
14	Antonysamy	57	M	Pus	SUR	Bed Sore	S	S	R	S	R	R	S	S	S	S							
15	Veeramani	47	M	Sputum	MED	Pneumonia	S	S	S	S	S	S	S	S	S	S							
16	Nagamani	43	M	Pus	SUR	Raw area-left thigh	S	S	R	R	R	R	S	S	S	S							
17	Sherlin	9	Fch	Pus	ORTHO OPD	# Distal femur	S	S	S	R	S	S	S	S	S	S							
18	Palanisamy	88	M	Urine	URO OPD	UTI	S	S	S	R	R	S	S	S	S	S	S	R	R				
19	Dalsan	4	Mch	Urine	PAED OPD	UTI	S	S	S	R	R	S	S	S	S	S	S	S	R				
20	Vasantha	42	F	Pus	ORTHO	Osteomyelitis	S	S	R	R	R	S	S	S	R	R							
21	Manickam	22	M	Pus	SUR	Cellulitis	R	R	R	R	R	S	R	S	R	R							
22	Palanisamy	71	M	Pus	MED	Bed sore	S	S	R	R	S	S	S	S	S	S							
23	Premalatha	25	F	Pus	OG/PN	Wound Gaping	S	S	S	R	S	S	S	S	S	S							
24	Saraswathy	51	F	Pus	SUR	Post MRM withSSG	S	S	R	R	R	S	R	S	R	R							
25	Rangan	62	M	Urine	URO OPD	UTI	S	S	R	R	R	R	R	R	R	R	R	R	R	N	N	P	P
26	Sanjai	9	Mch	Pus	SUR	Crush Injury	S	R	R	S	R	R	S	R	S	S				N	N	N	N
27	Seema	22	F	Pus	SUR	Post Op Wound Infection	R	R	R	R	R	R	R	S	R	R							

28	Ravindran	53	M	Pus	SUR	Diabetic Foot Ulcer	S	S	S	R	R	S	S	S	S	S							
29	Kamala	36	F	Pus	SUR	Raw area-left thigh	R	R	R	R	R	R	R	R	S	S				P	P	P	P
30	Palanisamy	58	M	Sputum	MED	Pneumonia, Old PT	S	S	S	R	S	S	S	S	S	R							
31	Anbalagan	59	M	Pus	MED	Necrotising Fascitis	S	S	R	R	R	S	S	S	S	S							
32	Gurusamy	63	M	Sputum	MED	Right lung Pneumonia	S	S	R	S	S	R	S	S	S	R							
33	Manikandan	22	M	Pus	SUR	Necrotising Fascitis	S	S	S	R	S	S	S	S	S	S							
34	Marimuthu	56	M	Pus	SUR	Gangrene Right foot	S	S	R	R	R	S	S	S	S	R							
35	Ramakrishnan	43	M	Urine	URO	UTI	S	S	R	R	R	R	R	S	S	S	R	R	R				
36	Murugesan	27	M	Pus	ORTHO	Crush Injury	S	S	S	R	R	S	S	S	S	R							
37	Ramasamy	41	M	Sputum	URO	Pneumonia	S	S	R	R	R	S	S	S	R	S							
38	Velayudham	74	M	Pus	SUR	Bed Sore	S	S	S	R	S	S	S	S	S	S							
39	Stephen	27	M	Pus	ENT	Traumatic rupture of Right TM	S	S	S	R	R	S	S	S	S	S							
40	Nagarathinam	46	F	Pus	SUR	Diabetic Foot Ulcer	S	S	S	R	S	R	S	S	S	S							
41	Surendar	11	Mch	Pus	SUR	Injection Site Abscess	S	S	S	R	S	S	S	S	S	S							
42	Nanjappan	81	M	Sputum	MED	Right lung Pneumonia	S	S	R	S	S	R	S	S	S	S							
43	Sabari	4	Fch	Urine	PAED ICU	Nephrotic Syndrome	S	S	R	R	S	R	S	S	S	S	S	S	R				
44	Swaminathan	41	M	Pus	SUR	Raw area-left leg	R	R	R	R	R	R	R	S	R	R							
45	Palani	52	M	Pus	ORTHO	Osteomyelitis	R	R	R	S	R	R	R	S	R	S							
46	Chandran	25	M	Pus	ORTHO	Ulnar Bursitis	R	R	S	R	R	S	R	S	R	R							
47	Surya	12	Mch	Pus	SUR	Post Op Wound Infection	R	R	S	S	R	S	R	S	S	S							
48	Periyasamy	62	M	Pus	SUR	Diabetic Foot Ulcer	S	R	R	R	R	R	R	R	R	S				N	P	N	P
49	Easwaran	29	M	Pus	SUR	Post Op Wound Infection	S	S	S	S	S	S	S	S	S	S							
50	Navomi	65	F	Pus	SUR	Bed sore	R	R	R	R	R	R	R	R	R	S				P	P	P	P
51	Manikandan	22	M	Pus	SUR	Raw area Rt Leg	S	R	R	S	R	R	S	S	S	R							
52	Krishnammal	42	F	Sputum	OG	Right lung Pneumonia	S	S	S	R	S	S	S	S	S	S							
53	Dineshkumar	17	M	Pus	ORTHO	# BB Left Forearm	S	R	R	R	R	R	R	S	R	R							
54	Ponnusamy	58	M	Sputum	MED	Pneumonia,Old PT	S	S	S	R	S	S	S	S	S	S							
55	Devi	22	M	Pus	SUR	Gangrene Right foot	S	R	R	S	S	R	S	S	S	S							
56	Surendar	6	Mch	Pus	ENT	ASOM	S	R	S	S	S	S	S	S	S	S							
57	Meenakshi	29	F	Pus	ORTHO	# Bimalleolar	R	R	R	R	R	R	R	S	R	R							
58	Maniraj	28	M	Sputum	MED	Pneumonia	S	R	S	S	R	S	S	S	S	S							
59	Seetha	44	F	Pus	SUR	Post MRM withSSG	S	S	S	R	R	R	S	S	R	S							
60	Dhanabal	51	M	Pus	SKIN	Psoriasis	S	S	S	R	R	R	S	S	S	S							

61	Subramani	71	M	Pus	SUR OPD	? Actinomycetes	S	S	S	R	S	R	S	S	S	S							
62	Valliyammal	71	F	Pus	ORTHO	# Right Femur	R	S	R	R	R	S	R	S	R	R							
63	Nandhu	8	Mch	Pus	SUR	Cellulitis	S	R	R	R	S	S	R	S	S	R							
64	Subramani	52	M	Pus	MED	PT with Right hydropneumothorax	S	R	S	S	R	S	S	S	S	S							
65	Savithri	41	F	Sputum	MED	Pneumonia, Old PT	S	R	S	S	R	S	S	S	S	S							
66	Palani	51	M	Pus	ORTHO	Crush Injury	R	R	S	R	R	R	R	S	R	R							
67	Murugan	57	M	Pus	ORTHO	#Tibia with Osteomyelitis	S	R	S	S	S	S	S	S	S	S							
68	Rajamanikam	52	M	Pus	SUR	Post Op Wound Infection	S	R	S	R	S	R	R	S	S	S							
69	Thiruthiya	3	Fch	Urine	PAED ICU	Nephrotic Syndrome	S	R	S	S	S	R	S	S	S	R	S	S	R				
70	Appasamy	37	M	Urine	URO OPD	UTI	S	R	S	S	R	S	S	S	S	S	S	R	R				
71	Rajendran	38	M	Pus	ORTHO	#Tibia with Osteomyelitis	S	S	R	R	R	R	S	S	R	R							
72	Vishwaraj	36	M	Pus	ORTHO	Crush Injury	S	R	S	S	R	S	S	S	S	S							
73	Dhanabal	58	M	Pus	SKIN	Psoriasis	S	R	S	S	R	S	S	S	S	S							
74	Sangeetha	11	Fch	Pus	SUR	Bed sore	S	R	S	S	R	S	S	S	S	S							
75	Gomathy	17	F	Cath Tip	URO	Septicemia	R	R	R	R	R	R	R	R	R	R				P	P	P	P
76	Banumathy	61	F	Pus	SUR	Post MRM withSSG	S	S	S	R	S	S	S	S	S	S							
77	Vellammal	78	F	Pus	ORTHO	# Femur	R	R	S	S	S	R	R	S	S	S							
78	Mahalingam	57	M	Urine	URO OPD	Urinary Calculous	S	R	R	R	R	R	R	S	S	R	R	R	R				
79	Lingaraj	42	M	Urine	URO	UTI	S	R	S	R	S	S	S	S	S	S	S	R	R				
80	Swaminathan	41	M	Pus	SUR	Post Op Wound Infection	R	R	S	R	R	R	R	S	R	R							
81	Palaniyammal	72	F	Pus	ORTHO	# Neck of femur	S	S	S	R	S	S	S	S	S	S							
82	Pushpa	61	F	Pus	SUR	Diabetic Foot Ulcer	S	S	R	R	S	R	S	S	R	R							
83	Mariammal	62	F	Urine	MED	Anaemia	S	R	S	S	S	S	S	S	S	S	S	R	R				
84	Subhathal	74	F	Sputum	MED	Pneumonia	S	R	S	S	S	S	S	S	S	S							
85	Ramasamy	61	M	Pus	SUR	Diabetic Foot Ulcer	S	R	S	S	R	S	S	S	S	S							
86	Mani	72	M	Pus	SUR	CaTongue, Hemiglossectomy	S	R	S	S	S	S	S	S	S	S							
87	Kannapan	35	M	Pus	ORTHO	Crush injury-Rt Lower limb	S	R	R	R	R	R	R	S	S	R							
88	Kanniyammal	32	F	Pus	SUR OPD	Ulcer Rt Foot	R	R	S	R	R	S	R	S	R	R							
89	Rajamanikam	51	M	Pus	SUR	Raw area-Post cellulitis	R	R	S	R	R	R	R	S	R	S							
90	Velumani	55	M	Pus	SUR	DM,BK Amputation	S	R	S	R	R	R	R	S	R	R							
91	Savithri	62	F	Sputum	MED	Pneumonia	S	R	S	S	R	S	S	S	S	S							

92	Ansar	72	M	Pus	SUR	DM,AK Amputation	S	R	S	S	S	S	S	S	S	S							
93	Balrubeen	56	M	Pus	ORTHO	# BB Rt Leg	S	R	S	S	R	S	S	S	S	S							
94	Angammal	65	F	Pus	ORTHO	Osteomyelitis	S	R	S	S	R	S	S	S	S	S							
95	Velusamy	57	M	Pus	SUR	BK Amputation	S	R	R	R	R	R	R	S	S	S							
96	Durai	72	M	Pus	ORTHO	# Neck of femur	S	R	R	R	R	S	R	S	S	S							
97	Kerthivasan	13	M	Pus	SUR	Cellulitis	S	S	R	R	R	S	R	S	S	S							
98	Sheik Mohammed	75	M	Pus	SUR	Raw area Cellulitis	S	S	S	S	R	S	S	S	S	S							
99	Sundaram	27	M	Pus	SUR ICU	Septic shock	S	S	S	S	R	S	S	S	S	S							
100	Chinnayan	71	M	Pus	SUR	Raw area Rt Forearm	S	S	S	S	S	S	S	S	S	S							
101	David	76	M	Pus	ORTHO	# Neck of femur	S	S	R	R	R	R	S	S	S	R							
102	Karthigeyan	25	M	Pus	SUR	Cellulitis	R	R	R	R	R	S	R	S	R	S							
103	Rathinasamy	65	M	Sputum	MED	Old PT,Pneumonia	S	S	S	S	S	S	S	S	S	S							
104	Rengathal	75	F	Pus	ORTHO	# Neck of femur	S	S	R	R	S	R	S	S	S	S							
105	Somaiya	31	F	Pus	OG/Gynaec	Pos Op Wound Infection	S	S	S	S	R	S	S	S	S	S							
106	Kalamani	45	F	Pus	SUR	Necrotising Fascitis	S	S	R	R	R	S	S	S	S	S							
107	Palani	57	M	Pus	SUR	Cellulitis Lt LowerLimb	S	S	R	R	R	S	R	S	S	S							
108	Omana	47	F	Pus	SUR	Non healing Venous Ulcer	S	S	S	R	R	S	R	S	S	S							
109	Nagarathinam	48	F	Pus	SUR	Diabetic Foot Ulcer	S	S	S	R	R	S	R	S	S	S							
110	Murugan	38	M	Pus	BURNS WD	20% BURNS	S	R	S	R	R	S	R	S	R	S							
111	Karthik	34	M	Pus	BURNS WD	30% BURNS	S	R	R	R	R	R	R	S	R	S							
112	Logeswaran	13	M	Pus	SUR	Necrotising Fascitis	S	S	S	R	R	S	S	S	R	S							
113	B/O Durga devi	2/365	Mch	Blood	NICU	Septicemia	S	S	S	R	R	S	S	S	S	S							
114	B/O Rani	1/365	Mch	Blood	NICU	Septicemia	S	S	S	S	S	S	S	S	S	S							
115	Manikandan	29	M	Urine	URO	UTI	R	R	S	R	S	S	R	S	R	S	R	R	R				
116	Santhosh	6	Mch	Urine	PAED ICU	Nephrotic Syndrome	S	S	S	R	S	R	S	S	S	S	S	S	R				
117	Ramsanth	11	Mch	Blood	PAED ICU	Septicemia	S	S	S	S	R	S	S	S	S	S							
118	B/O Malliya	1/365	Fch	Blood	NICU	Septicemia	S	S	S	S	R	S	S	S	S	S							
119	Durgadevi	8	Fch	ET Tube	PAED ICU	Septicemia	S	R	R	S	R	R	S	S	S	S							
120	Kaleshwari	2	Fch	Urine	SUR	Rectourethral Fistula	S	S	S	S	R	S	S	S	S	S	S	R	R				
121	Revathy	24	F	Pus	OG	LSCS-Wound Gaping	S	S	S	S	R	S	S	S	S	S							
122	Palaniyammal	61	F	Pus	ORTHO	# Neck of femur	S	R	R	R	R	R	R	S	R	S							

123	Kannapan	35	M	Pus	ORTHO	Crush Injury	S	S	S	S	R	S	S	S	S	S							
124	Ponnusamy	37	M	Pus	SUR	Gangrene Left foot	S	S	S	R	R	S	S	S	S	S							
125	Kamrudeen	38	M	Pus	ORTHO	Crush injury	R	S	R	S	R	R	S	S	S	S							
126	Viswanathan	58	M	Pus	SUR	Cellulitis Rt LowerLimb,CRF	R	S	R	S	R	S	R	S	S	S							
127	Palaniyappan	75	M	Pus	SUR	Nonhealing Ulcer Lt Foot	R	S	S	S	S	S	S	S	S	S							
128	Duraisamy	58	M	Sputum	MED	DM,COPD,Pneumonia	R	S	R	S	S	S	R	S	S	S							
129	Raman	81	M	Pus	MED	Bed sore/# Rt femur	R	S	S	S	R	S	S	S	S	S							
130	Hariharan	5	Mch	Aural swab	ENT	CSOM	S	S	S	S	R	S	S	S	S	S							
131	Balakrishna	35	M	Pus	ORTHO	Osteomyelitis	R	S	S	S	R	S	S	S	S	S							
132	Raja	27	M	Pus	ORTHO	# Humerus	S	S	R	R	R	R	S	S	S	S							
133	Rajeshkanna	41	M	Pus	SUR	RTA,AK Amputation	S	S	S	S	R	S	S	S	S	S							
134	Ambika	31	F	Pus	ORTHO	# BB Lt Leg	S	S	S	S	R	S	S	S	S	S							
135	Madhankumar	17	M	Urine	MED	UTI	S	S	R	R	R	R	S	S	S	R	S	R	R				
136	Martin	71	M	Urine	MED	Fever for Evaluation	S	S	S	S	R	S	S	S	S	S	S	R	R				
137	B/O Thangamani	1/365	Mch	Blood	NICU	Septicemia	S	S	S	S	R	S	S	S	S	S							
138	Banupriya	22	F	Blood	OG/LW	Septicemia	S	S	S	S	R	R	S	S	S	S							
139	Boopathy	2	Mch	Blood	PAED ICU	Septicemia	S	S	S	S	R	S	S	S	S	S							
140	Hariprasath	3	Mch	Urine	PAED ICU	Nephrotic Syndrome	S	S	S	S	R	S	S	S	S	S	S	S	R				
141	Anandkumar	31	M	Pus	ORTHO	# Humerus	S	S	S	S	R	S	S	S	S	S							
142	Dhandapani	45	M	Pus	IMCU	Ventilator Associated Pneumonia	S	S	R	S	R	S	R	S	S	S							
143	Vishalini	4	Fch	Aural swab	ENT	Bilateral Parotitis, ASOM,PLHA	S	S	S	S	R	S	S	S	S	S							
144	Kannapan	35	M	Pus	ORTHO	Open wound Lt Arm	S	S	S	S	R	S	S	S	S	S							
145	Vegappan	71	M	Pus	ORTHO	BK Amputation	S	S	S	S	R	S	S	S	S	S							
146	Mathiyalagan	21	M	Pus	ORTHO	Open wound Rt Forearm	R	S	R	R	R	R	R	R	R	S				P	P	P	P
147	Madhumitha	9	Fch	Urine	PAED	Fever for Evaluation	S	S	S	S	R	S	S	S	S	S	S	S	R				
148	Ramchander	66	M	Urine	URO OPD	UTI	S	S	S	S	R	S	S	S	S	S	S	R	R				
149	Aarthi	12	Fch	Pus	ORTHO	OPEN #Rt Forearm	S	S	S	S	R	S	S	S	S	S							
150	Sundara moorthy	45	M	Pus	ORTHO	# Humerus	S	S	S	S	R	S	S	S	S	S							
151	Laxmi	65	F	Pus	ORTHO	BK Amputation	S	S	S	S	S	R	S	S	S	S							
152	Gopalsamy	32	M	Pus	SUR	Diabetic Foot Ulcer	S	S	S	S	S	R	S	S	S	S							

153	Raman	71	M	Sputum	ORTHO	COPD	S	S	S	S	S	S	S	S	S	S								
154	Simon	36	M	Sputum	MED	Pneumonia	S	S	S	S	R	S	S	S	S	S								
155	Rangasamy	35	M	Pus	ORTHO	Osteomyelitis	S	R	S	S	R	R	S	S	S	S								
156	Ranganayaki	35	F	Sputum	MED	Pneumonia	R	S	S	S	S	S	S	S	S	S								
157	Kuppusamy	45	M	Pus	ORTHO	# BB Lt Leg	R	S	S	S	R	S	S	S	S	S								
158	Balachandran	22	M	Sputum	SUR	Pneumonia	R	S	R	S	R	R	R	S	S	S								
159	Mageswari	37	F	Aural swab	ENT	Rt CSOM With Central Perforation	S	R	S	R	R	S	R	S	R	R								
160	Seenusamy	62	M	Blood	MED	Septicemia	R	S	S	S	R	S	S	S	S	S								
161	B/O Suganya	1/365	Mch	Blood	NICU	Septicemia	R	S	S	S	R	S	S	S	S	S								
162	B/O Vinodha	2/365	Fch	Blood	NICU	?Sepsis	R	S	S	S	R	S	S	S	S	S								
163	B/O Kavitha	4/365	Mch	Blood	NICU	Neonatal Jaundice	R	R	S	S	R	S	R	S	R	S								
164	B/O Hema latha	3/365	Mch	Blood	NICU	Septicemia	R	S	S	S	R	S	S	S	S	S								
165	Krishnaveni	65	F	Pus	SUR	Toilet Mastectomy, Wound Infection	R	S	S	S	S	S	S	S	S	S								
166	Krishnasamy	63	M	Pus	SKIN	Leprosy-Trophic ulcer	S	S	R	R	R	R	S	S	S	S								
167	Selvam	49	M	Pus	SUR	Cellulitis with Necrotising Fascitis Rt Leg	R	S	R	S	S	R	R	S	S	S								
168	Savithri	65	F	Sputum	MED	Old PT,Pneumonia	R	S	S	S	S	S	S	S	S	S								
169	Arumugam	18	M	Sputum	MED	Pneumonia	R	S	S	S	R	S	S	S	S	S								
170	Kaliyammal	51	F	Sputum	OG/Gynec	Pneumonia	R	S	S	S	R	S	S	S	S	S								
171	Jaganathan	65	M	Pus	SUR	Nonhealing Venous Ulcer with HT	S	R	R	R	R	R	S	R	R	S				N	N	N	P	
172	Kuppamal	65	F	Sputum	ORTHO	PolyTrauma with Rt Hemothorax	S	S	S	R	R	S	R	S	S	S								
173	Krishnasamy	72	M	Pus	ORTHO	Wound Infection	S	R	S	S	S	S	S	S	S	S								
174	Jegadhambal	59	F	Urine	MED	UTI	S	S	S	R	S	S	S	S	S	S	S	R	R					
175	Rajini	26	M	Pus	BURNS WARD	4% Burns	R	R	R	S	R	R	S	R	R	R				P	P	P	P	
176	Sundara moorthy	45	M	Pus	ORTHO	Crush Injury	R	S	S	S	R	S	R	S	S	S								
177	Vegappan	71	M	Pus	ORTHO	# BB Rt Leg	R	S	S	S	R	S	R	S	S	S								
178	Narmadha	11	Fch	Pus	PAED ICU	Pyothorax	S	R	S	S	R	S	R	S	S	S								
179	Praneeth	10	Mch	Blood	PAED ICU	Nephrotic Syndrome	R	S	S	S	S	S	S	S	S	S								
180	Selvarani	4	Fch	Blood	PAED ICU	Septicemia	R	S	R	S	S	S	S	S	S	S								

181	Kalamani	45	F	Pus	SUR	DM,Necrotising Fascitis, Raw area Lt thigh	S	R	R	R	R	R	R	R	R	R				P	P	P	P
182	Kamalam	65	F	Pus	OG/Gynaec	Post Op Wound Infection	R	R	R	S	R	S	R	S	R	S							
183	Angel	23	F	Pus	OG/PN	LSCS Wound discharge	R	S	S	S	S	S	S	S	S	S							
184	Thambi Prasad	7	Mch	Blood	PAED ICU	Septicemia	R	S	S	S	R	S	S	S	S	S							
185	Shadikraj	2	Mch	Blood	PAED ICU	Septicemia	R	S	S	S	S	S	S	S	S	S							
186	Chitra	19	F	Pus	BURNS WARD	70% Burns	R	R	R	S	R	R	R	R	R	R				P	P	P	P
187	Nagaraj	39	M	Pus	ORTHO	Osteomyelitis Rt Femur	R	S	S	S	R	S	S	S	R	R							
188	Dhanalaxmi	22	F	Pus	OG/PN	LSCS Wound discharge	S	R	R	S	R	R	R	R	R	S				P	P	P	P
189	B/O Nivedha	1/365	Fch	Blood	NICU	Septicemia	R	S	S	S	R	S	S	S	S	S							
190	Kamrudeen	37	M	Pus	ORTHO	BK Amputation	R	R	R	S	R	R	R	R	R	R				P	P	P	P
191	Ponnusamy	65	M	Sputum	MED	Pneumonia,Old PT	R	S	S	S	S	S	S	S	S	S							
192	Ramasamy	55	M	Pus	SUR	Venous Ulcer	R	S	S	S	S	S	S	S	S	S							
193	Alagesan	45	M	Pus	SUR	Gangrene Left foot	S	R	R	R	R	R	R	S	R	S							
194	Prema	31	F	Sputum	MED	Pneumonia	R	S	S	S	R	S	S	S	S	S							
195	Mayilsamy	49	M	Sputum	MED	COPD	S	S	S	R	R	R	R	S	S	S							
196	Marykutty	54	F	Pus	MED	Bed sore/Paraplegia	R	S	S	S	R	S	S	S	S	S							
197	Anandhan	28	M	Sputum	MED	Pneumonia	R	S	S	S	R	S	S	S	S	S							
198	Archana	2	Fch	Blood	PAED ICU	Septic shock	R	S	S	S	S	S	S	S	S	S							
199	Balaiyan	53	M	Pus	SUR	SSG Site	R	R	R	R	R	R	R	S	R	S							
200	Muthusamy	49	M	Pus	ORTHO	Osteomyelitis	S	S	S	R	R	R	S	S	R	S							
201	Dhana pandiyan	53	M	Sputum	MED	COPD	R	S	S	S	R	S	S	S	S	S							
202	Janaki	72	F	Pus	ORTHO	#Distal 3rd Rt Femur	S	R	R	S	R	R	S	S	R	S							
203	Uma maheshwari	52	F	Urine	URO	UTI	R	S	S	S	S	S	S	S	S	S	S	R	R				
204	Bagyalaxmi	71	F	Sputum	MED	Pneumonia	R	S	S	S	S	S	S	S	S	S							
205	Muthusamy	44	M	Pus	SUR	Cellulitis	R	R	R	R	R	R	R	R	R	R				P	P	P	P
206	Kamrudeen	37	M	Pus	ORTHO	RTA,AK Amputation	R	S	R	R	R	R	R	R	R	R				P	P	P	P
207	Thangaraj	49	M	Pus	ORTHO	RTA, # Tibia	R	S	R	R	R	R	R	R	R	R				P	P	P	P
208	Rajmohan	25	M	Pus	SUR	SSG Site	R	S	S	S	R	S	S	S	S	S							
209	Karupammal	62	F	Pus	ORTHO	Diabetic Foot Ulcer	R	S	S	S	R	S	S	S	S	S							
210	Vijayakumar	14	M	Pus	SUR	Cellulitis	R	S	S	S	R	S	R	S	S	S							
211	Sarath	9	Mch	Pus	SUR	Cellulitis	S	R	S	S	R	R	S	S	R	S							

viii) KEY TO MASTER CHART

M	Male
F	Female
Mch	Male child
Fch	Female child
SUR	Surgery
MED	Medicine
OPD	Out Patient Department
ORTHO	Orthopaedics
PAED ICU	Paediatric Intensive Care Unit
NICU	Neonatal Intensive Care Unit
IMCU	Intensive Medical Care Unit
S	Sensitive
R	Resistant
P	Positive
N	Negative
BK	Below Knee
AK	Above Knee
RTA	Road Traffic Accident
CRF	Chronic Renal Failure
MRM	Modified Radical Mastoidectomy
Post op	Post Operative
SSG	Split Skin Graft
COPD	Chronic Obstructive Pulmonary Disease
#BB	Fracture Both Bones

LRI	Lower Respiratory tract Infections
HT	Hypertension
Ak	Amikacin
Gen	Gentamicin
Tob	Tobramycin
Cip	Ciprofloxacin
Caz	Ceftazidime
Cfs	Cefaperazone Sulbactum
Cpm	Cefipime
Mrp	Meropenem
Atm	Aztreonam
Pit	Piperacillin Tazobactum
Of	Ofloxacin
Nx	Norfloxacin
Nit	Nitrofurantoin